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ON THE FETAL MASCULINIZING ACTION OF CERTAIN ORAL PROGESTINS*

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ABSTRACT

Data are presented on 18 females with congenital masculinization of the external genitalia who were born of mothers treated with certain oral progestins during pregnancy. Nine of the mothers had received 19-nor-17α-ethynyltestosterone; 1, norethynodrel; and 8 others, 17α-ethynyltestosterone. The masculinizing effect on the female fetus of the first compound, and possibly the second, was comparable to that of known androgens. Labioscrotal fusion was exhibited only in those instances in which the oral progestin had been administered prior to the thirteenth week of gestation. Evidence is reviewed which suggests that these oral progestins have a direct masculinizing action on the female fetus. It is suggested that the discordance in androgenic activity exhibited by these compounds in the fetus as compared to the adult may be attributed to differences in the sensitivity of the end-organs and to delayed *fetal* disposal of steroid transferred across the placenta. The importance of distinguishing fetal masculinization due to administration of testosterone analogues with progestational activity from those rare instances of congenital masculinization of the female fetus in which a relationship to treatment during pregnancy with progesterone or its analogues or diethylstilbestrol has been suspected but not established, is emphasized. The authors recommend that treatment of pregnant women with semi-synthetic oral progestins which exhibit masculinizing properties in the *female fetus* be abandoned.

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THE association of female pseudohermaphrodism with the administration to mothers of 17-α-ethynyltestosterone (ethisterone, pregneninolone) or progesterone during pregnancy has been pointed out by Wilkins *et al.* (1) and by Jones (2), and was the subject of some discussion at the 1957 Laurentian Hormone Conference (3). At present, the frequency of this association and the importance of these preparations as virilizing agents in the female fetus are matters of controversy. It has been suggested that the masculinizing action may be due to an abnormality in the maternal metabolism of these steroids or in their rate of transfer across the placenta (1).

We have studied 18 female children with masculinization of the urogenital sinus and/or the external genitalia. All were the offspring of mothers treated with oral progestins during pregnancy. Nine of the mothers had received 17-α-ethynyl-19-nortestosterone (Norlutin); 1, norethynodrel (Enovid); and 8 others, 17-α-ethynyltestosterone. In accord with results of animal experiments, the clinical evidence favors the hypothesis of a direct androgenic action of these steroids on the female fetus.

OBSERVATIONS

The pertinent data on the 18 patients are shown in Table 1. Fusion of the labioscrotal folds (Fig. 1) was observed only in cases in which the mother had been treated prior to the thirteenth week of pregnancy. Treatment later in gestation was associated with clitoral enlargement (Fig. 2) but not with an appreciable degree of labioscrotal fusion. In 6 cases, mild and transitory signs of virilization were produced in the mother; these were present for the most part during the period of administration of the oral progestins. In only 1 instance did deepening of the voice persist for more than three months post partum. The comparative androgenic potency of the 3 oral progestins in the fetus can be estimated from the data of Wilkins *et al.* (1) and from the data contained in Table 1. The androgenic action of Norlutin and possibly of Enovid on the female fetus seems appreciably greater than that of 17-α-ethynyltestosterone, and is comparable to that of methyltestosterone (4). In 7 of the 18 patients, the mother had also been given other steroids. Stilbestrol in 5 of these cases should be considered as a possible factor contributing to the fetal masculinization (5). All of the patients with a low birth weight were born before the thirty-fifth week of pregnancy.

DISCUSSION

The masculinizing effects on the urogenital sinus and on the external genitalia of the female fetus of androgenic hormones administered to pregnant animals have been firmly established in placental mammals, including the monkey, from the results of a large number of experiments (4). In man, the androgen-induced forms of female pseudohermaphrodism (3) provide

TABLE 1

TABLE 1

Patient			Maternal hormonal therapy			External genitalia		Urinary 17-KS (mg./24 hrs.)	Sex chromatin pattern	Signs of virilization in mother
Name	Age	Birth wt. (Gm.)	Preparation*	Dosage	Time in pregnancy¶	Clitoral enlargement	Labioscrotal fusion#			
G.L.	4 days	3060	Norlutin	15 mg./day 10 mg./day 5 mg./day	10th–22nd week 22nd–34th week 34th–36th week	2.0 cm. ×1.0 cm.	+	0.2†	Female	None
A.R.	4 mos.	3070	Norlutin	40 mg./day 20 mg./day	7th– 8th week 8th–27th week	1.8×0.7 cm.	+++	0.8†	Female	None
S.B.	Newborn	1230	Norlutin	15 mg./day 5 mg./day	8th–18th week 18th–29th week	2.1 cm. ×0.7 cm.	+++	0.9†	Female	None
			Stilbestrol	75 mg./day	8th–29th week					
B.W.	Newborn	1500	Norlutin	10 mg./day 15 mg./day	7th–11th week‡ 11th–29th week	++	+++	—	Female§	Deepening of voice; increased facial hair
			Stilbestrol	25 mg./day 50 mg./day 75 mg./day 175 mg./day	9th–11th week 11th–13th week 13th–21st week 21st–29th week					
B.B.	6 days	2830	Norlutin	15 mg./day	7th–24th week	1.8 cm. ×0.8 cm.	++	0.9	Female	None
S.Bl.	13 days	2330	Norlutin	15 mg./day 40 mg./day 20 mg./day	5th– 9th week 9th–31st week 31st–35th week	1.4 cm. ×0.8 cm.	+++	—	Female	Deepening of voice; increased facial hair
			Premarin	3.75 mg./day 10.0 mg./day 5.0 mg./day	5th– 9th week 9th–31st week 31st–35th week					

* Norlutin: 19-nor-17α-ethynyltestosterone.
Enovid: A 10-mg. tablet contains: 9.85 mg. of 17α-ethynyl-17-OH-5(10)-estren-3-one and 0.15 mg. of ethynylestradiol 3-methyl ether.
Pranone, Lutocylol: 17α-ethynyltestosterone.
Proluton: Progesterone.
Delaluton: 17α-hydroxyprogesterone-17n-caproate.
¶ Dated from 2 weeks after last menstrual period.
# Labioscrotal fusion: + = fusion posteriorly but with a separate visible vaginal and urethral orifice.
++ = single slit-like or funnel shaped perineal orifice.
+++ = fusion extending to base or shaft of phallus.
† Urinary pregnanetriol <0.2 mg./24 hrs.
‡ Timed ovulation.
§ Postmortem examination showed a urogenital sinus, ovaries, uterus and Fallopian tubes; adrenal glands unremarkable.

(*continued on page 1372*)

TABLE 1—*continued*

Patient			Maternal hormonal therapy			External genitalia		Urinary 17-KS (mg./ 24 hrs.)	Sex chromatin pattern	Signs of virilization in mother
Name	Age	Birth wt. (Gm.)	Preparation*	Dosage	Time in pregnancy¶	Clitoral enlargement	Labioscrotal fusion#			
T.J.	9 wks.	1300	Norlutin	40 mg./day 20 mg./day	6th–19th week 19th–28th week	1.8 cm. ×0.6 cm.	+	—	Female	None
D.W.	2 days	2000	Norlutin	10 mg./day	15th–31st week	1.9 cm. ×0.8 cm.	0	0.3†	Female	Acne; increased facial hair; deepening of voice; ?slight clitoral enlargement
			Delalutin	250–500 mg./wk., i.m.	7th–32nd week					
S.M.	5 mos.	1250	Norlutin	10 mg./day	19th–28th week	2.5 cm. ×1.3 cm.	0	0.5†	Female	None
			Stilbestrol	75 mg./day 50 mg./day	13th–19th week 19th–28th week					
			Proluton	100 mg./o.1 d. ×8, i.m.	13th week					
A.S.	5 wks.	2990	Enovid	10 mg./day	6th–38th week	1.8 ×0.8 cm.	++	0.2†	Female	Acne; increased facial hair; deepening of voice
H.N.‖	3 yrs.	2760	Pranone	200 mg./day	2nd–22nd week‡	2.9 cm. ×1.0 cm.	++	1.5†	Female	None
			Stilbestrol	5–75 mg./day	6th–38th week					
D.McC.	5 yrs.	1350	Lutocylol	30 mg./day	6th–32nd week	3.0 cm. ×0.8 cm.	+	2.2†	Female	Acne; increased facial hair
S.H.	4 yrs.	3780	Pranone	200 mg./day 100–150 mg./day 200–250 mg./day 75 mg./day	4th– 8th week‡ 8th–12th week 12th–33rd week 33rd–36th week	3.4 cm. ×1.2 cm.	++	1.8†	Female	Deepening of voice
			Stilbestrol	15–25 mg./day 50 mg./day	7th–13th week 13th–36th week					
B.N.	Fetus	about 15 wks. gestation	Pranone	30 mg./day	11th–15th week	++	+++	—	Female§	None
D.F.	8 yrs.	3275	Pranone	?	7th–38th week	3.7 cm. ×1.8 cm.	++	3.3†	Female	None
S.S.	10 mos.	1920	Pranone	100 mg./day 50 mg./day	4th– 5th week 5th–36th week	+	++	0.5	Female	None
L.W.	5 yrs.	3100	Pranone	50 mg./day	8th–12th week	++	0	0.8	Female	None
B.S.	Newborn	1100	Pranone	45 mg./day	16th–28th week	1.5 cm.	0	0.5	Female	None

‖ Normal male twin; no evidence of a blood group chimera.

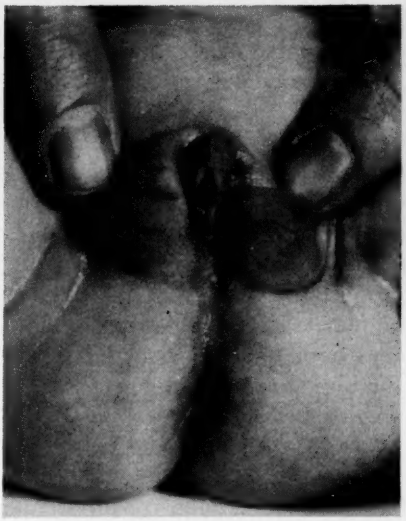

FIG. 2. D.W., aged 10 days. Clitoral enlargement without significant labioscrotal fusion.

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FIG. 1. The appearance of the external genitalia of S.B. at age 3 months, illustrating the enlarged clitoris, labioscrotal fusion, and prominent, fleshy labia majora.

evidence that exposure of the female fetus to hormones with androgenic activity from either maternal or fetal sources can inhibit downward descent of the uterovaginal "complex" and induce varying degrees of masculine differentiation of the urogenital sinus and the external genitalia. Recently, 14 cases of iatrogenic female pseudohermaphrodism due to the administration of testosterone or its analogues to pregnant women have been reviewed by Grumbach and Ducharme (4). The clinical data in these cases indicated the importance of the dose and potency of the androgen, the length of administration, and especially the time in gestation during which the fetus was exposed, in determining the degree of fetal masculinization. Labioscrotal fusion with a urogenital sinus was present in this group only in those instances in which the female fetus had been exposed to androgen prior to about the thirteenth week of pregnancy. The critical importance of the age of the fetus and the limited period of sensitivity of the urogenital sinus to the action of androgens has been clearly demonstrated by Jost (6–8) and others (9, 10) in experimental animals. In the series of patients reported here and in that of Wilkins *et al.* (1) there was a similar correlation between the time in gestation that the fetus was first exposed to certain oral progestins and the extent of masculinization of the external genitalia. In general, the degree of fetal masculinization is less than that seen in female pseudohermaphrodites with congenital virilizing adrenal hyperplasia, although there is also considerable variation in the appearance of the external genitalia at birth in the latter disorder. In many, but not all, of the

‖ Normal male twin; no evidence of a blood group chimera.

patients with iatrogenically produced female pseudohermaphrodism, the extent of labioscrotal fusion was disproportionately greater than the degree of clitoral enlargement. Contrariwise, newborn female infants with congenital adrenal hyperplasia usually show clitoral enlargement proportional to, or greater than, the degree of labioscrotal fusion.

This discordance may be partly attributable to the fact that in iatrogenic cases there is a limited period during pregnancy in which the various steroids are administered to the mother, in contrast to the prolonged exposure of the fetus to adrenal androgens in congenital adrenal hyperplasia, as well as to the dose and potency of the androgen. An abnormal configuration of the external genitalia in newborn female infants following administration to pregnant women of hormones with androgenic activity may easily be overlooked in a cursory examination of the external genitalia. In 2 of this group of patients and in 1 previously reported (4), the genital abnormality, although quite prominent, had been overlooked for several years.

In considering the mechanism of fetal masculinization by progestins, it is necessary to distinguish clearly the possible mode of action of the analogues of testosterone which exhibit progestational activity from the action of progesterone and its analogues. Masculinization of the female fetus resulting from the administration of hormones during gestation could result from an indirect action of these preparations on the genital tract of the fetus. This might involve an unusual metabolic pathway in the mother or fetus serving to transform these steroids to androgens; or it might stem from their influence on a maternal or fetal endocrine gland such as the hypophysis, adrenal cortex or ovary, which could lead secondarily to the secretion of excessive androgen.

However, it seems more probable that the oral progestins, 17-α-ethynyltestosterone, 17-α-ethynyl-19-nortestosterone, and norethynodrel (17-ethynylestrenolone) have a direct androgenic effect on the female fetus. Although no direct evidence is available on the placental transfer of these steroids, Salhanick (personal communication) has shown that labeled testosterone administered to a woman during the twelfth week of pregnancy rapidly passes into the fetal circulation and tissues. In 1942, Courrier and Jost (11) reported that the administration of 17-α-ethynyltestosterone[1] to pregnant rabbits caused masculinization of the female fetus. This finding subsequently was confirmed in 1947 by Jost (12) in additional experiments. Similar experiments with the newer oral progestins have not been reported.

[1] It is of interest that this steroid was called pregneninolone by Inhoffen and Holweg because of its lack of an androgenic effect on the comb of the capon and its ability to produce a secretory endometrium in the mature castrated rat and rabbit. However, unlike progesterone, it does not maintain pregnancy in castrated placental mammals.

Lyster *et al.* (13) showed that testosterone analogues with potent progestational activity have comparatively weak androgenic activity when assayed in castrated rats. Similarly, these substances when administered to nonpregnant women have rarely produced signs of virilization (14, 15), though Wilkins *et al.* (1) observed such an effect in a sexually infantile girl treated with 20 mg. of Norlutin daily for three months. However, there appears to be a definite difference in the androgenic properties of these oral progestins in the fetus as compared with the nonpregnant woman. It has already been shown that 17-α-methylandrostenediol dipropionate has a potent androgenic action on the rat fetus, although it is a comparatively weak androgen in the adult rat (16).

The discrepancy in the androgenic properties of these oral progestins in the adult as compared with the fetus could be attributed to differences in the sensitivity of end-organs (8, 17) or to delayed *fetal* disposal of steroid transferred across the placenta.[2] There is evidence to support the latter notion. Alterations in hepatic function and intrahepatic cholestasis have been described following the administration of methyltestosterone (19, 20) and 17-α-ethyl-19-nortestosterone (Nilevar) (21, 22). Modifications of the testosterone molecule comparable to those in certain oral progestins delay their rate of catabolism by the liver (23–25). In addition, Jost (16) noted that the androgenic activity of the 17-α-methyl derivative of androstenediol was appreciably greater than that of androstenediol itself in the fetal rat in contrast to the adult rat. Accumulating evidence in both animals and man indicates that certain enzyme activities are absent or deficient in the liver of the fetus and of the newborn infant (26–30). For example, it has been shown recently that the rate of disposal of hydrocortisone is delayed in some premature and full-term newborn infants (31, 32). Further, the concentrations of progesterone and estriol in fetal plasma are considerably higher than in maternal plasma at term (33). Pregnanediol has not been detected in the urine of newborn infants even after the injection of progesterone (34). These observations, in the light of the clinical evidence, suggest that the androgenicity of certain oral progestins in the fetus is related to differences in fetal metabolism, particularly the rate of disposal of these steroids.

It is noteworthy that the testosterone analogues with progestational activity do not maintain pregnancy in the ovariectomized rat or rabbit (35, 36). Furthermore, administration of these substances to intact pregnant rats and rabbits results in a high incidence of abortion or fetal resorption (12, 36).

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[2] A delay in maternal disposal, similar to that noted for hydrocortisone, may also be of importance (18).

The fact that testosterone and its analogues exhibit varying degrees of progestational activity on the endometrium of various species of placental mammals including man is not unique (37–40). Certain of these analogues also have inherent estrogenic activity (41–43). An overlap in the activities of a variety of steroids is well known; recently Hertz and Tullner (44) have shown that certain steroid-17-spirolactones which act as antagonists of aldosterone and desoxycorticosterone have a progestational property.

In regard to the practical implications of the clinical observations, there is an important question not yet satisfactorily answered: What is the incidence of masculinization of female fetuses following administration of these oral progestins to pregnant women? The available data are insufficient to permit even a crude approximation. It has already been pointed out that unless abnormalities of the external genitalia are specifically sought in female infants, they may readily be overlooked. Although 17-α-ethynyltestosterone has been used for the treatment of habitual and threatened abortion for many years, fetal masculinization has not been observed frequently. On the other hand, there is evidence that the androgenic action of ethynylnortestosterone (and possibly norethynodrel) on the fetus, as well as its progestational activity, is considerably greater.[3] Indeed, ethynylnortestosterone is comparable in its androgenic activity to methyltestosterone. Moreover, these substances have been used only recently in the treatment of pregnant women and, in our limited experience, the incidence of associated genital anomalies seems appreciably greater than with 17-α-ethynyltestosterone. Wilkins (personal communication) has studied 4 similar patients whose mothers had been given ethynylnortestosterone during pregnancy, and 4 additional cases have been detected in other clinics.

The role of individual variation in sensitivity to the action of androgens must also be considered. Vandekerckhove (46) has described a woman with carcinoma of the breast who received large amounts of methylandrostenediol for a prolonged period during pregnancy. Neither the mother nor her female infant exhibited any signs of virilization. In addition, there are a few reports of non-adrenal female pseudohermaphrodites who have not had associated renal or cloacal anomalies, or a history of exposure to a known source of androgen during gestation (1, 3). We have studied 3 additional cases of this syndrome at the Babies Hospital. The anomalous development of the external genitalia in the latter simulated the configuration of the genital tract in androgen-induced female pseudohermaphrodism. It

[3] The oral progestin, 19-nor-17α-methyltestosterone, appears to have similar properties. Recently, Carpentier (45) described a stillborn female fetus with masculinized external genitalia, the offspring of a mother who had ingested 10 mg. of this compound daily between the twelfth and twenty-third week of pregnancy.

seems possible that during gestation these patients also were subjected to excess androgen from some maternal or fetal source. In any event, such cases are uncommon and in our experience have been significantly less frequent than instances of fetal masculinization associated with the administration during pregnancy of certain C_{19} oral progestins.

The use of these preparations in pregnant women for the treatment of threatened or habitual abortion must be questioned in the light of the clinical evidence. It is no longer permissible to believe that the administration of these steroids during pregnancy carries an insignificant risk to the fetus. There is, it is true, an effective surgical operation for the repair of the genital abnormality. However, only a few surgeons have had an extensive experience with the plastic procedure and we are aware of some instances in which the reconstruction of the external genitalia was quite unsatisfactory, and of others complicated by an infection of the urinary tract. Dr. John Lattimer of the Department of Urology has developed a procedure for clitoral recession in patients without adrenal hyperplasia in whom clitoral enlargement presents a problem, so that amputation of the clitoris can be avoided. In a few patients, the enlarged clitoris has spontaneously diminished in size during infancy. Nevertheless, the genital abnormality is iatrogenic and is clearly an avoidable and unnecessary complication of gestation.

It is the responsibility of pharmaceutical manufacturers to provide evidence that semi-synthetic oral progestins do not exhibit androgenic activity in the fetus of pregnant experimental animals before these preparations are released for general use. More consideration must be given to the physiologic and biochemical individuality of the fetus and newborn infant, and of the possible toxic effects (47) on the fetus of drugs administered to pregnant women. It is also the responsibility of those who advocate the treatment of complications of pregnancy with certain oral progestins which have fetal masculinizing properties, to prove their efficacy. Although such evidence is difficult to obtain, it must be more than anecdotal in nature. We can only conclude that treatment of pregnant women with C_{19} steroids possessing progestational properties and exhibiting androgenic activity in the female fetus should be abandoned. The treatment of pregnant women with progestins should be limited 1) to compounds not injurious to the fetus, and 2) to those capable of maintaining pregnancy.

The evidence that associates progesterone therapy with congenital masculinization is tenuous. Albeit, this hormone when injected in large doses has a weak androgenic action on certain end-organs of castrated male animals (13, 48, 49); such an effect has not been observed in pregnant experimental placental mammals. Four partially masculinized female infants have been described (1, 50) whose mothers received progesterone during

pregnancy. In 1 instance the mother also received methyltestosterone (1). In 2 other cases (1) stilbestrol in large amounts had also been given. Recently, the latter compound has been implicated by Bongiovanni *et al.* (5) as a possible fetal masculinizing agent when administered in large doses to some pregnant women. The only instance in which progesterone alone was given to the mother was in the case of the female infant described by Hayles and Nolan (50). During the tenth week of pregnancy the mother received a total of 30 mg. of progesterone as three 10-mg. injections of a repository preparation (Lutocyclin, Ciba). This amount is less than the estimated endogenous secretion of progesterone at this stage of gestation. It is possible that the administration of large amounts of progesterone to *certain* pregnant women may result occasionally in masculinization of the female fetus. However, this relationship has not yet been established. It is important that the fetal masculinizing properties of the testosterone analogues with progestational activity be not categorized with those of progesterone and its analogues, nor with that of diethylstilbestrol.

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THE INFLUENCE OF ESTROGEN ON THE SECRETION, DISPOSITION AND BIOLOGIC ACTIVITY OF CORTISOL*

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ABSTRACT

The following findings were obtained which bear on the mechanisms concerned in the rise of plasma free 17-hydroxycorticoid (17-OHCS) levels in patients treated with estrogenic hormone (ethinyl estradiol or diethylstilbestrol): 1) Cortisol accounted for approximately 80 per cent of the plasma free 17-OHCS in a normal subject given estrogen. 2) In patients treated with estrogenic hormone there was a marked rise in the level of plasma free 17-OHCS but no increase in urinary total 17-OHCS excretion. 3) In a man with intact adrenals who received ACTH intravenously and in an Addisonian woman who received cortisol intravenously there was a greater rise in the level of plasma free 17-OHCS and urinary F+E but a lesser rise in urinary THF+THE when estrogen was also administered. 4) On three occasions when patients with adrenal insufficiency were given cortisol intravenously, estrogen produced a diminished rate of removal of free 17-OHCS from plasma and a smaller distribution volume of the injected F. 5) The pituitary glands of 3 patients who had been receiving long-term estrogen therapy showed no Crooke's changes in the basophil cells. It is concluded that the rise in the level of plasma cortisol in patients receiving estrogen is due mainly to a diminished transformation of compound F to certain metabolites and to a greater retention of the hormone within the intravascular or possibly the extracellular fluid compartment. The failure of patients treated with estrogens to show evidence of an excess of cortisol is believed to be due in part to a failure of tissue levels of F to rise to the same degree as the plasma level of the hormone.

THE administration of ethinyl estradiol or diethylstilbestrol produces a rise in the level of plasma free[1] 17-hydroxycorticoids which exceeds that observed in many cases of Cushing's syndrome (1, 2). Nevertheless, during long-term estrogen therapy (*e.g.*, in patients with carcinoma of the

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[1] The term "free" refers to the nonconjugated compounds rather than to those 17-hydroxycorticoids which are not bound to plasma proteins.

prostate) Cushing's syndrome does not develop. In an attempt to resolve these apparently incompatible findings, studies have been made of the secretion, disposition and biologic activity of cortisol (compound F) in patients receiving estrogenic hormone.

Factors affecting the biologic activity of endogenous cortisol which might be influenced by estrogen are illustrated in Figure 1. The phrase "biologic activity of endogenous cortisol" is meant to indicate the ultimate biologic activity which results from interaction of such factors as the secre-

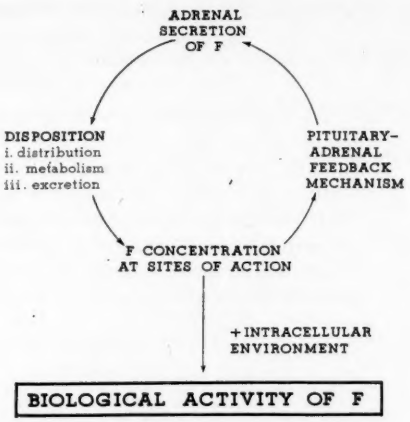

FIG. 1. Factors affecting the biologic activity of endogenous cortisol (F).

tion and disposition of the hormone and the state of the intracellular environment in which the hormone is believed to act. For example, the "biologic activity of endogenous cortisol" might be increased as a result of one or more of the following changes: an increased secretion of the hormone, an alteration in its disposition such that more hormone reaches the tissues, or an increased sensitivity of the intracellular environment to a given amount of hormone. In Figure 1 it has been assumed that the concentration of F at its sites of action (probably within the cells) is a more accurate index of what its biologic activity is likely to be than is the plasma level of the hormone.[2] For example, the assumption is made that it is the concentration of F within the cells of the tissue controlling pituitary ACTH production which regulates the pituitary-adrenal feedback mechanism, rather than the concentration of plasma F per se. The supposition is also made that cortisol itself is the active form of the hormone reaching the intracellular environment.

[2] The term plasma cortisol, as used throughout this paper, refers to cortisol both bound and unbound to plasma proteins (3).

MATERIALS AND METHODS

All clinical studies were carried out in the Clinical Investigation Unit. The patients with carcinoma of the prostate showed no abnormality of renal function and no clinical or laboratory evidence of metastases. Except when stated to the contrary, none of these patients had undergone orchidectomy.

The estrogens used, ethinyl estradiol and diethylstilbestrol, were given by mouth in dosages which possessed less biologic activity than the amount of estradiol believed to be secreted during the third trimester of pregnancy (4, 5).

The methods employed for the determination of plasma free 17-hydroxycorticoids, urinary total 17-hydroxycorticoids, cortisol (F), cortisone (E), tetrahydrocortisol (THF) and tetrahydrocortisone (THE) have been described previously (6). It must be pointed out that with the chromatographic systems used it is probable that the "THE area" contained the allopregnane isomer of THF (7) as well as the pregnane isomer of THE, and the "THF area" contained only the pregnane isomer of THF. Urinary sodium and potassium were measured by flame photometry, urinary chloride by the method of Volhard and Harvey (8), and urinary glucose by the procedure of Froesch and Renold (9). Urinary creatinine (10) was determined in order to check the completeness of collections.

PROCEDURE AND RESULTS

I. *The nature of the plasma free 17-hydroxycorticoids (17-OHCS) in subjects receiving estrogenic hormone*

The method (11) used for the estimation of the plasma level of free 17-OHCS utilizes the Porter-Silber colorimetric reaction. This reaction is relatively specific for 17,21-dihydroxy-20-ketosteroids, a group which includes cortisol, cortisone, 11-desoxy-17-hydroxycorticosterone (Reichstein's compound S), and their dihydro and tetrahydro derivatives. It was important to determine the nature of the compounds measured by the free 17-hydroxycorticoid method in the plasma of subjects receiving estrogenic hormone. For this purpose a 30-year-old normal woman was given 0.1 mg. of ethinyl estradiol every six hours for six days during the first half of the menstrual cycle. The level of plasma free 17-OHCS rose from a control value of 20.4 μg., to 41.8 μg. per 100 ml. on the sixth day of estrogen administration. On this sixth day, a sample of blood (approximately 375 ml.) was withdrawn into 120 ml. of dextrose-citrate solution. The citrated plasma was separated and duplicate 5-ml. samples were taken for measurement of free 17-OHCS by the Peterson procedure (11). The remainder of the citrated plasma was processed according to this procedure. The purified methylene-chloride extract was taken to dryness and further purified by a benzene-water partition. The water was evaporated to dryness and the residue dissolved in methanol and chromatographed on paper for forty-eight hours, using the toluene-propylene glycol system. The blue tetrazolium reaction was seen in 2 spots on the chromatogram. When the effluent from this chromatogram, and the benzene from the benzene-water parti-

tion, were chromatographed on paper using the benzene:methanol:water (2:1:1) system, no blue tetrazolium-reacting material was detected. The *first* blue tetrazolium-reacting spot on the toluene-propylene glycol chromatogram was considered to be cortisol, for the following reasons. It gave the Porter-Silber reaction and it showed absorption when the chromatogram was photographed using an ultraviolet lamp which had over 95 per cent of its output at 256 mμ. Furthermore, the unknown substance showed the same running rate as an authentic sample of cortisol, both before and after acetylation (12), in the toluene-propylene glycol system. The *second* blue tetrazolium-reacting spot on the original chromatogram had the same running rate as an authentic sample of cortisone but the substance was not characterized further. When the compounds presumed to be cortisol and cortisone were eluted and estimated by the Porter-Silber reaction, using cortisol as the standard, the results (expressed in μg. per 100 ml. of the citrated plasma) were as follows: (a) free 17-OHCS as determined on the citrated plasma by the Peterson method—24.6 μg.; and (b) 17-OHCS eluted from the chromatogram—cortisol 18.8 μg., and "cortisone" 2.9 μg. It is concluded that cortisol accounted for approximately 80 per cent of the elevated plasma free 17-OHCS level in this subject receiving ethinyl estradiol.

II. *The influence of estrogen on the secretion of cortisol*

It was decided to determine first whether subjects treated with estrogen show a diurnal variation in plasma free 17-OHCS levels. For this purpose, determinations were made at appropriate times during the day and night on 3 men with carcinoma of the prostate. At the time of the study, the first patient (B.B., 75 years old) had been taking 1 mg. of diethylstilbestrol daily at 10 A.M. for over five years; the second (A.T., 78 years old) had been taking 1 mg. of this hormone approximately every eight hours for over eighteen months; the third (W.C., 71 years old) had been receiving 5 mg. of diethylstilbestrol approximately every twelve hours for over a year. The plasma free 17-OHCS values along with the urinary total 17-OHCS values are illustrated in Figure 2. For comparison, similar studies are shown on a 38-year-old normal male subject (J.L.) and on a 35-year-old man with Cushing's syndrome (W.W.) who was subsequently found at operation to have bilateral adrenal hyperplasia. In the men receiving estrogen there was a diurnal variation in the level of plasma free 17-OHCS similar in pattern to that observed in the normal subject (13). However, for the larger part of the 24-hour period, the levels were above the normal range[3] for the

[3] This normal range was derived from determinations on blood samples drawn between 8 A.M. and 10 A.M. In view of the diurnal variation in plasma free 17-OHCS concentration, it is likely that the normal range of values will show a similar variation throughout the day and night.

period 8 A.M. to 10 A.M. Despite this elevation, these 3 patients showed no signs of Cushing's syndrome and their urinary excretion of total 17-OHCS was within the normal range. In contrast, in the patient with Cushing's syndrome (W.W.), the urinary total 17-OHCS and the plasma free 17-OHCS level were increased, and the latter showed little or no diurnal variation. The foregoing findings indicate that in patients treated with estrogen the normal diurnal variation in cortisol secretion is maintained. They further show that estrogen produces an increase in the concentration of plasma free 17-OHCS which is not accompanied by a proportionate increase in urinary total 17-OHCS excretion. This phenomenon was also observed in the following 2 studies.

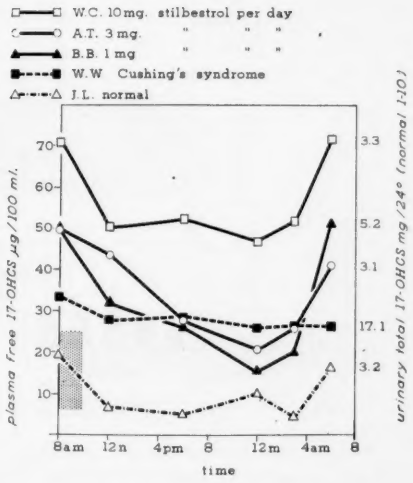

FIG. 2. Diurnal pattern of plasma free 17-OHCS levels in 3 patients with carcinoma of the prostate receiving diethylstilbestrol therapy (W.C., A.T. and B.B.), in 1 man with Cushing's syndrome (W.W.), and in 1 normal male (J.L.). The dotted rectangular area on the left depicts the 8–10 A.M. normal range for plasma free 17-OHCS. The urinary total 17-OHCS values are shown alongside the final point of the individual curves. (The curve on Patient W.C. has been published previously by the authors (6).)

Figure 3 depicts the daily course of plasma free 17-OHCS and urinary total 17-OHCS levels in a 48-year-old man with carcinoma of the prostate who was given 0.1 mg. of ethinyl estradiol every six hours for seven days. It can be seen that there was a rapid rise in the plasma free 17-OHCS level to values well above the normal range, but there was no increase in the urinary total 17-OHCS excretion. A similar rise in the concentration of plasma free 17-OHCS was observed in a 19-year-old patient with gonadal dysgenesis who was given as little as 0.5 mg. of diethylstilbestrol daily. Her morning plasma free 17-OHCS levels before treatment and following six and thirteen months of treatment were, respectively, 13.9, 47.5 and 34.8 µg. per 100 ml. At no time did this patient show signs of Cushing's syndrome. Similar findings have been reported by Wallace and her associates (2). Figure 4 illustrates the effect of ethinyl estradiol, in a dosage of 0.25 mg. every six hours, on the plasma free and urinary total 17-OHCS

levels in a patient with Cushing's syndrome (W.W. in Fig. 2). Towards the end of each of the 2 control periods and of the period of estrogen administration, a dose of 25 U.S.P. units of adrenocorticotropin (ACTH, Armour lot No. R-68909) was given intravenously in 500 ml. of 5 per cent dextrose in water over an eight-hour period (8 A.M. to 4 P.M.). On all but the days when the patient received ACTH, the level of plasma free 17-OHCS was determined on blood drawn at 8 A.M.; on the ACTH days the determinations were made on blood drawn at 8 A.M. and 4 P.M. It can be seen that when ACTH was given during each control period there was a

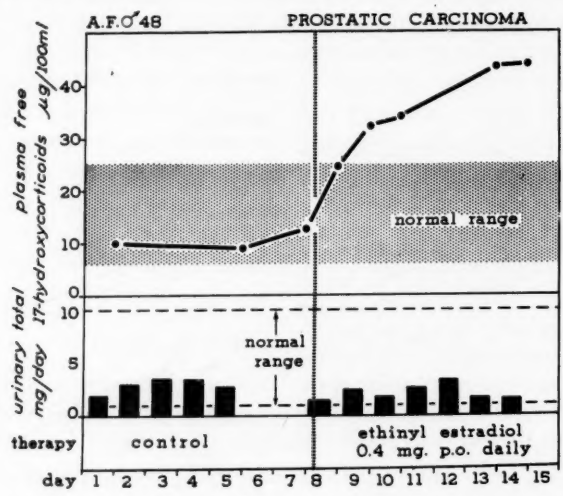

FIG. 3. The influence of ethinyl estradiol on the level of plasma free 17-OHCS and urinary excretion of total 17-OHCS in Patient A.F. Blood was drawn on each occasion at 8 A.M.

marked rise in both the plasma free and urinary total 17-OHCS values. In contrast, with estrogen alone (by the end of the period of its administration) the plasma free 17-OHCS levels rose *above* those achieved with ACTH alone in the control periods, but there was little increase in urinary total 17-OHCS excretion. Furthermore when ACTH was given during the period of estrogen administration, there was a greater response as determined by the rise in plasma free 17-OHCS levels, but no greater response as measured by the rise in urinary total 17-OHCS excretion.

Further study of the foregoing phenomenon was carried out in a male patient with apparently normal adrenals (A.F. in Fig. 3). This man was given ACTH during a control period and again on the eighth day of estrogen administration (ethinyl estradiol, 0.1 mg. every six hours) in the same

manner and using the same lot of ACTH as in the study on Patient W.W. Blood was drawn for determination of plasma free 17-OHCS at appropriate times before, during, and following the infusion of ACTH. Urines collected for appropriate periods were analyzed for their content of F, E, THF and THE. The results of this study are shown in Figures 5 and 6. It can be seen that the capacity to respond to the 25-unit dose of ACTH, as measured by a rise in the excretion of F+E+THF+THE, was no greater in the estrogen period than in the control period. However, the response to the ACTH as determined by the increase in the levels of urinary F+E and

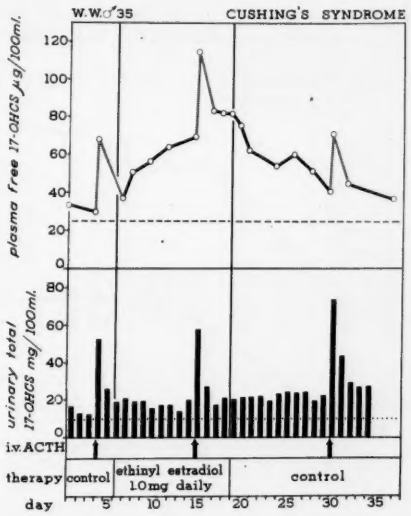

FIG. 4. The influence of ethinyl estradiol on the levels of plasma free 17-OHCS and urinary total 17-OHCS, with and without the administration of intravenous ACTH, in Patient W.W. Blood was drawn at 8 A.M. except on the ACTH days, when it was drawn at 8 A.M. and 4 P.M.

plasma free 17-OHCS was greater in the estrogen period than in the control period (the 8 A.M. to 4 P.M. increment in plasma free 17-OHCS was 42.6 μg. per 100 ml. during estrogen period and 32.5 μg. during the control period). A similar hyperactive response to ACTH (as measured by the rise in the concentration of plasma free 17-OHCS) in patients receiving ethinyl estradiol has already been reported by Wallace and her associates (2).

These findings are interpreted as follows. In patients receiving estrogenic hormone, the rise in the level of plasma free 17-OHCS without a measurable increase in urinary total 17-OHCS is not due, appreciably, to increased secretion of cortisol. Rather, it is due to an alteration in the disposition of cortisol which is at least partly the result of diminished transformation of the hormone to certain of its metabolites such as THF and THE (Fig. 6). It must be emphasized, however, that the urinary total 17-OHCS (F+E+THF+THE) account for only one quarter to one third of the

cortisol secreted by the adrenal cortex. Consequently, a failure of urinary total 17-OHCS excretion to rise or fall does not necessarily mean that there has been no change in cortisol secretion. For example, increased cortisol secretion in the presence of unchanged total 17-OHCS excretion could occur if there were an increased transformation of the 17-OHCS to their 20-hydroxy derivatives.

III. *The effect of estrogen on the disposition of cortisol*

Further evidence of an estrogen-induced alteration in cortisol disposition came from a study of plasma free 17-OHCS and urinary total 17-OHCS

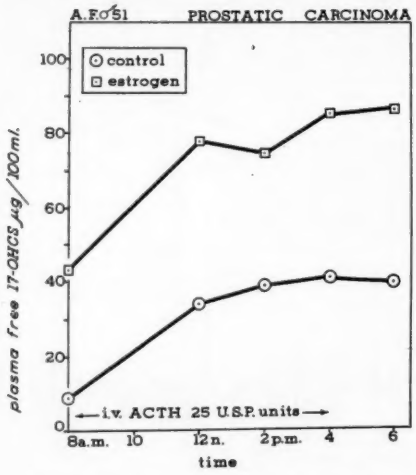

FIG. 5. The effect of ethinyl estradiol on the response of plasma free 17-OHCS levels to intravenous ACTH (25 U.S.P. units) in Mr. A.F.

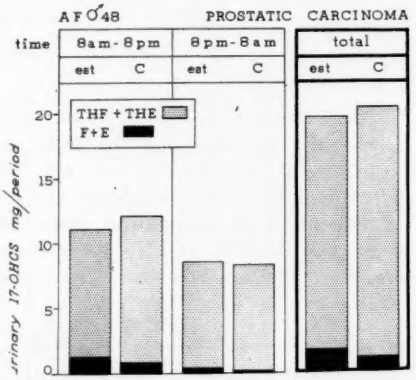

FIG. 6. Urinary excretion of F+E and THF+THE in Mr. A.F. following intravenous infusion of ACTH (25 U.S.P. units, 8 A.M. to 4 P.M.), before (control) and during the period of administration of ethinyl estradiol (est.).

following the administration of compound F to Addisonian patients. In the first study, Patient R.K., a 55-year-old woman with Addison's disease, was given 100 mg. of cortisol during a control period and also on the eighth day following the institution of estrogen therapy (0.1 mg. of ethinyl estradiol every eight hours). The cortisol was administered intravenously from 8 A.M. to 8.30 A.M. in 250 ml. of 5 per cent dextrose in water containing 10 ml. of ethanol. Plasma free 17-OHCS levels were determined at 8 A.M. and at appropriate intervals following the infusion. Urines were collected for appropriate periods and analyzed for the content of F, E, THF and THE. During the entire period of study, with the exception of the days on which the cortisol infusion was given (at which time oral cortisol was dis-

continued), the patient was maintained with cortisol, 10 mg. orally at 8 A.M. and 4 P.M., and 0.1 mg. of fluorohydrocortisone acetate orally at 8 A.M. Results are shown in Figures 7 and 8. It can be seen that when estrogen was given, a larger proportion of the administered cortisol was excreted unchanged and in the form of cortisone, and a smaller proportion was excreted as the metabolites THF and THE. This observation provides further evidence that administration of estrogen results in a diminished transformation of F to certain of its metabolites, such as THF and THE. In Figure 8 the plasma free 17-OHCS levels observed in this study are depicted. In the drawing of the curves it was assumed that distribution equilibrium of the administered cortisol had been reached by 10 A.M., ninety

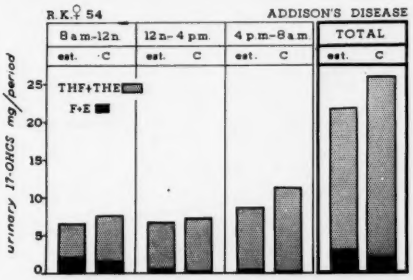

FIG. 7. Urinary excretion of F+E and THF+THE in Mrs. R.K. following intravenous infusion of 100 mg. of cortisol (8 to 8:30 A.M.), before (control) and during the period of administration of ethinyl estradiol (est.).

minutes following the completion of the infusion. Two findings are evident from Figure 8. Firstly, administration of estrogen resulted in a diminished rate of removal from plasma of the free 17-OHCS, as manifested by a prolongation of the half-life. This observation suggests diminished transformation of F to certain metabolites. Similar data in patients with *intact* adrenals have been reported by Wallace and her associates (2). Secondly, the zero intercepts on the graph indicated a value of 214 μg. per 100 ml. for the "estrogen" curve (patient's weight 73.6 Kg.) and 168 μg. per 100 ml. for the "control" curve (patient's weight 73.9 Kg.). This observation suggests that during estrogen therapy there was a smaller volume of distribution of the injected cortisol, perhaps due to a greater retention of F within the intravascular or possibly even the extracellular fluid compartment (14). The 8 A.M. value for plasma free 17-OHCS concentration was zero on the infusion day during the control period, and 7.3 μg. per 100 ml. on the infusion day during the period of estrogen administration. This finding is believed to reflect an estrogen-induced diminished metabolism of the oral cortisol given on the day prior to the cortisol infusion. It is noted that the half-life of plasma free 17-OHCS following the control infusion of F in this patient was already above the range reported for normal subjects (15). No explanation for this could be found.

A similar study was performed in the same Addisonian patient six months later. On this occasion a dose of 50 mg. rather than 100 mg. of cortisol was administered intravenously during a control period and also on the fourth day of estrogen administration (0.1 mg. ethinyl estradiol every six hours). Findings similar to those of the previous study were obtained. The half-times and zero intercepts were respectively 448 minutes and 170 μg. per 100 ml. for the "estrogen" curve (patient's weight 70.8 Kg.) and 202 minutes and 112 μg. per 100 ml. for the "control" curve (patient's weight 72.5 Kg.). The 8 A.M. values for plasma free 17-OHCS were 0.9 μg. per 100 ml. on the control infusion day, and 5.5 μg. per 100 ml. when the infusion was given during the period of estrogen administration.

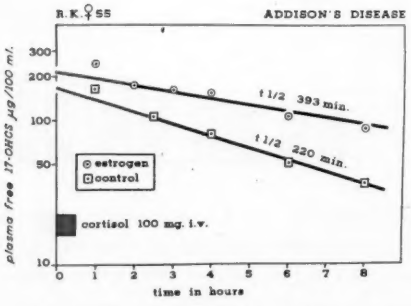

FIG. 8. Plasma free 17-OHCS levels following intravenous infusion of 100 mg. of cortisol to Patient R.K., before and on the eighth day of administration of ethinyl estradiol (0.1 mg. every eight hours).

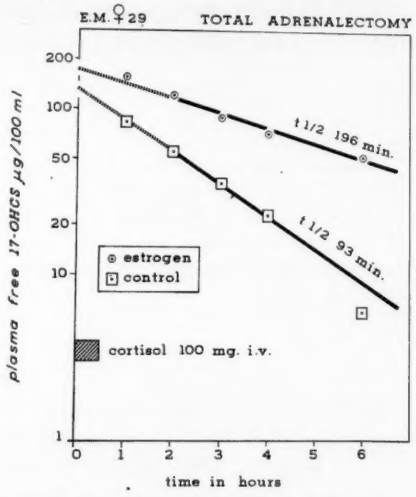

FIG. 9. Plasma free 17-OHCS levels following intravenous infusion of 100 mg. of cortisol to Patient E.M. before and on the eighth day of administration of ethinyl estradiol (0.1 mg. every six hours).

A third study of this type was carried out in a 29-year-old woman who had undergone total adrenalectomy for Cushing's syndrome (bilateral adrenal hyperplasia) four months previously. A dose of 100 mg. of cortisol was given intravenously during a control period and again on the eighth day of estrogen administration (0.1 mg. ethinyl estradiol every six hours). This patient was maintained with cortisol (20 mg. at 8 A.M. and 10 mg. at 3 and at 10 P.M.) and fluorohydrocortisone (0.1 mg. at 8 A.M.). On the day preceding each cortisol infusion day, the entire dose of 40 mg. of F was

given at 8 A.M. On the day of the infusion, the usual maintenance therapy was discontinued. From Figure 9 it can be observed that once again, during treatment with estrogen, there was a diminished rate of removal of free 17-OHCS from plasma following injection of cortisol. The zero intercepts indicated 172 μg. per 100 ml. for the "estrogen" curve (patient's weight 76.4 Kg.) and 132 μg. per 100 ml. for the "control" curve (patient's weight 76.0 Kg.). The 8 A.M. values for plasma free 17-OHCS were zero on the control infusion day and 4.8 μg. per 100 ml. on the infusion day in the estrogen period.

A possible interpretation of the foregoing findings is illustrated in Figure 10. In this schema it is assumed that estrogen exerts no *direct* effect on

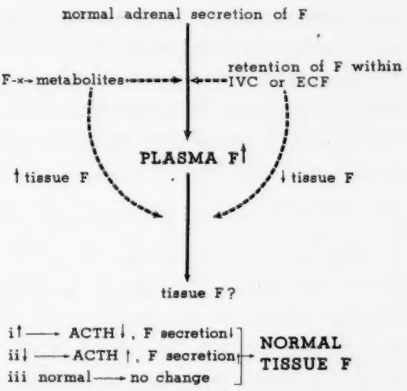

FIG. 10. Possible consequences of effect of estrogen on the disposition of cortisol (F); i, ii and iii indicate the 3 possible levels of tissue F.

the adrenal secretion of F. Estrogen administration, however, brings into play 2 factors which will alter the disposition of cortisol: 1) a diminished transformation of F to certain metabolites, and 2) a greater retention of F within the intravascular or possibly the extracellular fluid compartment. Both of these factors will operate to increase the plasma level of F. They will oppose each other, however, as far as determining what the tissue or intracellular level of F will be; the first factor will tend to make more F available for passage into the cells whereas the second factor, by retaining F within the plasma or extracellular fluid, will tend to limit the amount of F reaching the intracellular environment. What the resulting tissue level will be is uncertain. There are 3 possibilities: 1) this level may be increased, *although it would not be increased to the same degree as the plasma level of the hormone;* 2) it may be decreased; or 3) it may be normal. The first of these possibilities is favored for the following reasons. In the first study in Patient R.K. the amount of F appearing in the urine following intravenous cortisol was greater when estrogen was given (2.18 mg.) than when it was

not (1.64 mg.). If the level of urinary F parallels the level of unbound F in the plasma (*i.e.*, the F which is not bound to plasma protein) then it might be predicted that the estrogen-induced alterations in the disposition of cortisol would lead initially to an increase in concentration of unbound F in the plasma and of F in the tissues. Whatever this initial level of tissue F may be, if the pituitary-adrenal feedback mechanism functions normally, the secretion of pituitary ACTH and of adrenal F will alter in such a way that the level of F in the tissues will be restored to normal (Fig. 10). Thus equilibrium will be reached with a normal intracellular concentration of F but a high plasma concentration of F. An hypothesis such as this would explain how the plasma cortisol level could remain elevated in face of an intact pituitary-adrenal feedback mechanism. It could do so if the feedback mechanism were responding to changes in the level of intracellular F rather than to changes in the plasma level of the hormone. In this schema it is assumed that estrogen induces little or no alteration in the renal handling of cortisol. It is not assumed, because of possible differences in cell permeability to cortisol in different tissues, that the intracellular concentration of F will be the same for all tissues.

IV. *The influence of estrogen on the biologic activity of endogenous cortisol*

Whether or not Figure 10 proves to represent a correct interpretation of the foregoing findings, it is clear that the biologic activity of cortisol will be influenced not only by the concentration of F at its various sites of action, but also by the intracellular environment (Fig. 1). What is known of the biologic activity of endogenous cortisol (see Introduction) in patients who are receiving estrogenic hormone? Perhaps the best evidence on this point is clinical. Patients with carcinoma of the prostate who are being treated with estrogen do not show definite evidence of either Cushing's syndrome or Addison's disease. Hence it is unlikely, on the basis of these criteria, that the biologic activity of endogenous cortisol is *greatly* increased or decreased by the administration of estrogen.

There is further evidence of a pathologic nature on this question. With the help of Dr. C. Ezrin, the pituitary glands of 3 patients with carcinoma of the prostate who had been receiving long-term estrogen therapy were examined for the presence of Crooke's changes in the basophil cells. Pertinent details of the history of these patients are as follows:

The 3 patients were respectively 83, 71 and 59 years old. At the time of death the first patient had been receiving at least 1 mg. of diethylstilbestrol daily for twelve years; the second, 12 mg. of tri-para-anisylchloroethylene (TACE) daily for eight months; and the third, at least 3 mg. of diethylstilbestrol daily for fifteen months. Only the third patient had undergone orchiectomy. The first and third patients died of acute hemorrhage from the urinary tract. The clinical course in the second patient was one of gradual deterioration. Autopsy in the first patient revealed local tumor extension only;

in the second, there were extensive metastases in bone; and in the third, metastases were found in the lung, liver and bone marrow.

It is assumed that in these men the plasma free 17-OHCS levels were above normal. In none of the 3 were Crooke's changes or even degranulation found in the cytoplasm of the pituitary basophils (beta cells). Such changes are almost invariably present in the pituitaries of patients who have high levels of plasma free 17-OHCS as the result of Cushing's syndrome or following the administration of cortisone in greater than physiologic doses (16). Hence, insofar as Crooke's changes are a manifestation of excessive activity of cortisol, it seems unlikely that the biologic activity of endogenous cortisol was greatly enhanced in these 3 men who had been treated with estrogen for long periods.

A third approach to the investigation of the biologic activity of endogenous cortisol consisted of a study of the influence of estrogen on the effect of intravenous cortisol on urinary excretion of sodium, chloride, potassium and glucose in an Addisonian patient. During the entire period of study in the first intravenous cortisol experiment (Figs. 7 and 8), Mrs. R.K. was given a constant diet containing 171 milliequivalents of sodium and 75.9 milliequivalents of potassium. On the cortisol infusion days and on selected days prior to these days during both the non-estrogen and estrogen periods, urine was collected for appropriate periods and analyzed for content of sodium, chloride, potassium and glucose.

The results of this experiment are illustrated in Figure 11. The "control" values were obtained on days preceding the cortisol infusion day during both the non-estrogen and estrogen periods. On the days when no intravenous F was given, the administration of estrogen did not appear to alter the excretion of either electrolytes of glucose. When F was infused *during the control period* there was retention of sodium and chloride which became evident by the second period (12 noon to 4 P.M.) and disappeared, as far as sodium was concerned, during the last period (12 midnight to 8 A.M.). There was potassium diuresis which was still evident during the third period (4 P.M. to 8 P.M.); thereafter a rebound retention of potassium occurred. Increased excretion of glucose was seen during the period from 12 noon to 4 P.M. When F was infused *during the period of estrogen administration* there was a greater retention of sodium and chloride than that observed when the infusion was given during the control period. Little or no potassium diuresis was seen, however, and there was, if anything, a smaller response as measured by increase in urinary glucose. Thus despite the higher levels of plasma free 17-OHCS achieved when cortisol was infused during the estrogen period, there was no greater biologic effect as far as urinary potassium and glucose excretion were concerned, but there was a more marked effect as measured by sodium and chloride excretion.

One cannot draw firm conclusions from one experiment such as this. It is possible that the greater effect on sodium and chloride excretion might have been due to more cortisol reaching the appropriate sites of action. If, however, more cortisol reached the sites of action for potassium and glucose effect, then the somewhat smaller influence of intravenous cortisol on the excretion of these substances during the estrogen period might be

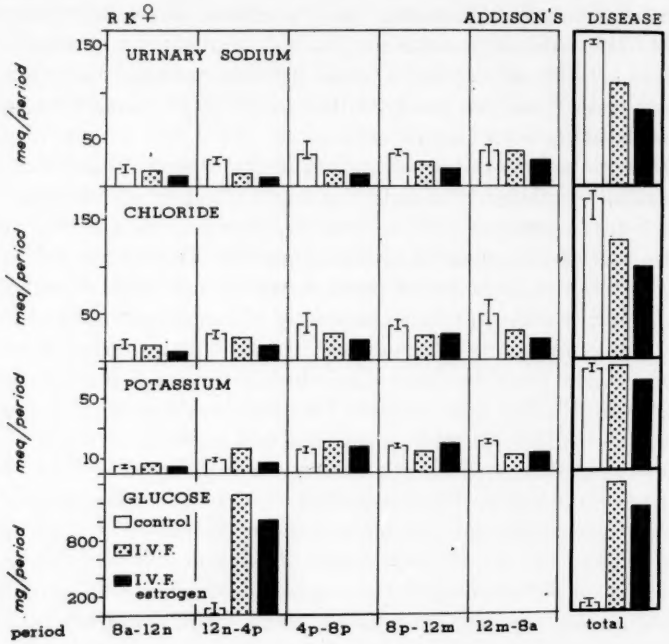

FIG. 11. The influence of ethinyl estradiol on the effect of intravenous cortisol (100 mg., 8 to 8:30 A.M.) on urinary excretion of sodium, chloride, potassium and glucose in Patient R.K. The values for urinary glucose are not recorded for the periods 8 A.M. to 12 noon, 4 P.M. to 8 P.M., 8 P.M. to 12 midnight and 12 midnight to 8 A.M. because in all instances (control, intravenous cortisol (I.V.F.), and I.V.F. with estrogen) they were below 50 mg. per period. For the control values the height of the block represents the mean, and the bar represents the range.

explained on the basis of an interference with cortisol activity within the cell. It should be pointed out, that the foregoing experimental situation was an artificial one in that it did not permit a direct or indirect (*i.e.*, through the pituitary-adrenal feedback mechanism, see Fig. 10) effect of estrogen on adrenal secretion of cortisol but only an effect on the disposition of the hormone and on the intracellular environment. Nonetheless, it did provide

a situation similar to that which might occur when estrogen is given to a patient receiving exogenous cortisol.

DISCUSSION

It should be emphasized that the method used for determination of plasma free 17-OHCS in all probability measures not only the cortisol which is unbound to plasma proteins but also that which is bound. Hence one does not know whether the estrogen-induced rise in the concentration of plasma free 17-OHCS represents an increase in bound cortisol, unbound cortisol, or both.

The observation that estrogen, though it produces a rise in the plasma level of cortisol, fails to produce an increase in urinary total 17-OHCS excretion has been interpreted in this report as indicating that estrogen does not cause an increase in cortisol secretion. However, one must await the application of more precise methods for measurement of cortisol secretion (17) before being certain of the interpretation given here. It must also be pointed out that the present experiments do not rule out an effect of estrogen on the renal handling of cortisol.

Whatever the effect of estrogen on the *secretion* or *excretion* of cortisol, it is evident that the estrogen-induced rise in the level of plasma cortisol is due, in part at least, to 2 alterations in the *disposition* of the hormone: 1) a smaller volume of distribution of cortisol, and 2) a diminished transformation of cortisol to certain of its metabolites. The findings with regard to distribution of cortisol have been interpreted as indicating a greater retention of cortisol within the intravascular or possibly even the extracellular fluid compartment. This phenomenon could be due to one or more of the following 3 factors: 1) an increased binding of cortisol to plasma proteins, 2) a decreased ability of the hormone to pass across capillary walls, and 3) a diminished permeability of tissue cell membranes to cortisol. In this regard, an increased binding of cortisol to certain plasma proteins has recently been reported in pregnant subjects (3), and in patients receiving estrogen (18).

The conclusion that in subjects treated with estrogen there is a diminished transformation of cortisol to certain of its metabolites is based on the finding that estrogen produced a greater rise in plasma and urinary levels of F but a smaller rise in urinary THE+THF, in response to ACTH in Patient A.F. and in response to intravenous cortisol in Patient R.K. This metabolic block could be located at the step of saturation of ring A of the cortisol molecule. Similar conclusions regarding estrogen and the metabolism of cortisol have been expressed by Wallace and her associates (2). That the apparent inhibition of cortisol metabolism is not necessarily the result of the alteration in distribution of the hormone is suggested by the

work of Glenn (personal communication), who observed inhibition of the degradation of cortisol by the rat-liver microsome supernatant fraction in the presence of estradiol.

The estrogen-induced disturbance in transformation of cortisol to certain of its metabolites is similar to that seen in patients with cirrhosis of the liver (19). Yet in cirrhotic patients the levels of plasma free 17-OHCS are normal, whereas in patients receiving estrogen they are increased. This discrepancy could be explained by the second alteration in cortisol disposition induced by estrogen, namely, the change in distribution of the hormone. If, as a result of this change, tissue cortisol levels failed to rise to the same degree as the plasma level of the hormone, then the pituitary-adrenal feedback mechanism might not respond sufficiently to restore the plasma cortisol level to normal.

It has been pointed out here that despite the rise in plasma cortisol concentration, patients with carcinoma of the prostate who undergo long-term estrogen therapy show little evidence of Cushing's syndrome and no Crooke's changes in the pituitary basophil cells. This phenomenon is also believed to be due largely to an alteration in the distribution of cortisol such that there may be failure of the tissue cortisol levels to increase to the same extent as the plasma level of the hormone. That there may be estrogen-induced changes in the intracellular environment which limit the actions of cortisol cannot be ruled out by the results of present studies.

As the data in this report are similar, qualitatively at least, to those described for the secretion, disposition and biologic activity of cortisol in pregnant women (6, 20), it would be reasonable to relate the latter findings to increased estrogen secretion. It is of interest that a rise in the concentration of plasma free 17-OHCS to levels above normal was obtained by the oral administration of as little as 0.5 mg. of diethylstilbestrol daily. This dose possesses biologic activity comparable to that of the amount of estradiol believed to be secreted during the menstrual cycle (4, 21). One wonders, therefore, whether alterations in disposition of cortisol qualitatively similar to those described in the present report may not occur in a cyclic fashion during the female reproductive period. Such considerations, however, must make allowance for a possible effect of progesterone on the secretion and disposition of cortisol.

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URINARY PREGNANETRIOL OF TESTICULAR ORIGIN*

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ABSTRACT

The daily administration of 5000 I.U. of chorionic gonadotropin to 9 men with functioning testes resulted in a rise in the urinary excretion of pregnane-3α,17α,20α-triol which averaged 1.3 mg. per day on the third and fourth days of treatment. This increase was significantly greater than the usual day-to-day variation. In 1 man with Addison's disease the elevation in pregnanetriol excretion was 1 to 1.5 mg. In a male eunuchoid and in 2 women (one ovariectomized and the other in the pre-ovulatory phase of the menstrual cycle), pregnanetriol excretion was not influenced by treatment with chorionic gonadotropin. These results indicate that the pregnanetriol increment was derived from the testes and not the adrenal cortex. A portion of the urinary pregnanetriol of normal men may accordingly be assumed to be of testicular origin. It is suggested that the testicular pregnanetriol was a metabolic derivative of 17α-hydroxyprogesterone, which is probably an intermediary in the biosynthesis of testosterone.

PREGNANE-3α,17α,20α-triol (pregnanetriol) has long been recognized as a urinary derivative of the adrenal cortex. It was first isolated by Butler and Marrian (1) in 1937 from the urine of women with the adrenogenital syndrome due to tumors or hyperplasia of the adrenals. These observations were subsequently confirmed (2, 3) and then re-emphasized by Bongiovanni *et al.* (4), who noted that large amounts of pregnanetriol were excreted with some regularity in the urine of patients with adrenal hyperplasia and virilism. It had been suggested (2) that this pregnanetriol was a metabolite of 17α-hydroxyprogesterone, and such a derivation from administered 17α-hydroxyprogesterone has been demonstrated by Langecker (5). After Hechter *et al.* (6) provided the first indication that 17α-hydroxyprogesterone was an intermediary in the biosynthesis of the adrenocortical hormones, it was proposed that the pregnanetrioluria of the adrenogenital syndrome reflected an accumulation of 17α-hydroxyprogesterone due to an enzymatic defect or deficiency in the C21 and C11 hydroxylating mechanisms (4).

With improved analytical techniques, small amounts (0.2–2.0 mg. per twenty-four hours) of pregnanetriol have now been found in the urine of

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normal men and women (7, 8). Although this could readily be explained on the basis of the liberation of small quantities of 17α-hydroxyprogesterone from the adrenals, the steroid hormone-secreting gonads must also be considered as a source of some of the pregnanetriol precursor. Several groups of investigators have demonstrated by *in vitro* experiments that the testes of both rats and humans synthesize androgen by a series of enzymatic reactions which seem to duplicate in part the adrenal biosynthetic process (9–12). The testes contain an enzyme which converts the Δ^5,3-hydroxy steroids (Δ^5-pregnenolone) to α,β-unsaturated ketones (progesterone) (9). The conversion of progesterone to 17α-hydroxyprogesterone and then to the secretory products, androstenedione and testosterone (13, 14), by the interstitial cells of Leydig has been indicated by these studies. The liberation or secretion of some 17α-hydroxyprogesterone during the synthesis and secretion of these testicular androgens could account for some of the pregnanetriol found in the urine of normal men.

To test this proposition, the effect of testicular stimulation with chorionic gonadotropin on the urinary excretion of pregnanetriol has been examined in a number of men.

METHODS

The subjects were 5 normal men, 4 men with spermatogenic defects but apparently normal rates of androgen secretion, a man with fully treated Addison's disease, a male eunuchoid, a woman who had been ovariectomized, and a normal young woman during the preovulatory phase of the menstrual cycle. All were ambulatory.

From all subjects, 24-hour collections of urine were obtained routinely for seven or eight days. After at least three days of baseline collection had been completed, a four-day course of chorionic gonadotropin[1] was started in a dosage of 5000 I.U. per day by intramuscular injection. Completeness of urine collections was checked by creatinine determinations (15). Aliquots of 24- to 48-hour urine pools during the control period were analyzed for pregnanetriol, pregnanediol and 17-ketosteroids. The same analyses were made on the fourth and sometimes the third 24-hour pool during the treatment period.

17-Ketosteroids were determined on a crude neutral extract of urine by a modification of the Holtorff-Koch procedure (16). Pregnanetriol and pregnanediol were measured on urine aliquots after glucuronidase hydrolysis and chromatographic separation of the urine extract on an alumina column into two fractions (one containing pregnanetriol and the other pregnanediol) according to the procedure of Bongiovanni and Clayton (8). The absorption spectra of sulfuric-acid chromogens were determined in both fractions. When the characteristic absorption spectrum of pregnanediol or pregnanetriol was not observed in the appropriate portion of the extract, it was assumed that an unmeasurably small quantity was present. Quantitative estimates of the amounts of pregnanetriol or pregnanediol were made on the basis of the corrected readings at 430 A° (8).

In several instances the purified extracts containing pregnanetriol were chromatographed on filter paper with formamide and methanol (50:50) as the stationary phase and benzene as the mobile solvent (17). Pregnanetriol and perhaps closely related

[1] Generously supplied by Dr. Edward C. Reifenstein, Jr. of E. R. Squibb & Sons, and Dr. John B. Jewell of Ayerst Laboratories.

alcoholic steroids were located on the paper strips by heating after the papers had been sprayed with 50 per cent trichloracetic acid in ethanol and dried (18). The rate of migration and the area and intensity of the fluorescent spots obtained from the extracts were compared with those of a known sample[2] of pregnane-3α,17α,20α-triol. From these comparisons visual estimates were made of the quantity of pregnanetriol in the purified extracts.

RESULTS

In the normal men, those with spermatogenic disorders, and the one with Addison's disease, the urinary excretion of pregnanetriol rose during the third and fourth days of chorionic gonadrotropin administration (Table 1). In individual cases the increase ranged from 0.8 to 3.1 mg. per day. The average elevation was 1.3 mg. daily, which is approximately double the mean control level of pregnanetriol excretion in this group of men. The increment was substantially greater than the usual variation noted from day to day under a relatively constant program of treatment, diet and activities, as noted here and in unpublished studies from this laboratory. In all but one of the men, urinary 17-ketosteroid excretion was also enhanced (3–14 mg. per day); in none was there a detectable quantity of pregnanediol, either before or during treatment with chorionic gonadotropin.

Urinary pregnanetriol excretion was not increased during treatment in the male eunuchoid, the ovariectomized woman or the normal woman in the preovulatory phase of her menstrual cycle.

When portions of the pregnanetriol-containing fractions of the extracts of urine from 5 of the men were chromatographed on paper as described, only spots which ran at approximately the same rate as pure pregnane-3α,17α,20α-triol were readily identified. When pregnane-3α,17α,20α-triol was added to the extract before its application, the size and intensity of the fluorescent area was increased, and there was no clear indication of a separation of 2 compounds with slightly different running rates. It should, however, be pointed out that these alcoholic steroids do not form as discrete spots during paper chromatography as do some ketonic steroids, and the tailing which was noted might have obscured the separation of closely related compounds such as pregnane-3α,17α,20β-triol. Aliquots of extracts containing 25 or 50 micrograms of pregnanetriol as measured spectrophotometrically were applied beside the same quantity of the pure compound,[2] and visual comparisons were made of the size and intensity of the fluorescent areas after chromatography. In every case the extract appeared to contain approximately the amount of pregnanetriol previously determined chemically. Samples of pregnane-3α,17α,20α-triol were not actually isolated and measured, but the results of these paper chromatographic anal-

[2] Purified pregnanetriol was donated by Merck Sharp & Dohme.

TABLE 1. EFFECTS OF FOUR-DAY COURSES OF 5000 UNITS PER DAY OF CHORIONIC GONADOTROPIN (CG) ON THE URINARY EXCRETION OF PREGNANE 3α, 17α, 20α-TRIOL (PREGNANETRIOL) AND 17-KETOSTEROIDS

Subject	Pregnanetriol (mg./24 hrs.)		17-Ketosteroids (mg./24 hrs.)	
	Control	3rd & 4th days of CG admin.	Control	3rd & 4th days of CG admin.
R.W.—Normal male	1.9, 2.1	3.3	15.9	21.3
L.T.—Normal male	0.9	1.8, 1.7	19.5	25.0
J.M.—Normal male	1.3, 1.1	2.1, 1.9	34.7, 34.2	41.4, 48.5
R.M.—Normal male	1.4	3.1, 2.6	28.3	40.1
R.K.—Normal male	0.9	2.4, 1.8	16.9	17.6, 17.4
R.I.—Oligospermia	0.6	2.0, 2.1	16.4	19.1
F.G.—Oligospermia	0.9	2.0, 2.1	13.6	19.0
P.G.—Oligospermia	1.1, 2.0	2.2, 4.3	14.3	19.9
E.B.—No germ cells	1.1	4.2	16.5	26.6
D.N.—Addison's disease (male)	0.3	1.8, 1.3	8.2	14.1
B.D.—Eunuchoid (male)	0.3, 0.3	0.1, 0.3	6.9	7.8
B.P.—Ovariectomized female	0.1, 0.2	0.1	—	7.0
J.W.—Pre-ovulatory normal female	1.7, 1.4	1.6, 1.5	—	—

yses lend strong support to the specificity of the spectrophotometric quantitative procedure.

DISCUSSION

The interstitial-cell stimulating capacity of chorionic gonadotropin in men has been well established. The resulting enhancement of the rate of testicular androgen secretion in men was indicated by a rise in the excretion of urinary 17-ketosteroids as shown here (Table 1) and the induction of an anabolic process like that induced by testosterone (19, 20). Chorionic gonadotropin exerts no such influence in men without functioning testes, indicating that these effects are not the result of adrenocortical activation (20, 21).

With this background it might well be assumed that the increment in urinary pregnanetriol induced by administration of chorionic gonadotropin was derived from the testes. The excretion of pregnanetriol was not increased by this treatment in the ovariectomized and the pre-ovulatory women nor in the male eunuchoid, but did rise from a control level of 0.3 mg. to 1.3 and 1.8 mg. per day in Subject D.N. (Table 1), the male Addisonian. It thus appears reasonably certain that the adrenal cortex did not contribute to the increase in urinary pregnanetriol.

As in the case of urinary pregnanetriol of adrenal origin, the pregnane-

triol derived from testes is probably a metabolite of 17α-hydroxyprogesterone. This steroid has been isolated from incubated testicular tissue, and has been proposed as an intermediary in the enzymatic transformation of Δ^5-pregnenolone to the testicular androgens, testosterone and androstenedione (9–12). A small proportion of 17α-hydroxyprogesterone might well escape during this biosynthetic process, as it apparently does from the adrenal cortex. The increased excretion of pregnanetriol induced by chorionic gonadotropin would accordingly reflect a greater rate of formation and output of testicular 17α-hydroxyprogesterone. This would suggest that the gonadotropin accelerates the rate of androgen secretion by either enhancing the rate of conversion of progesterone to 17α-hydroxyprogesterone or accelerating an antecedent biochemical reaction in the series leading to the synthesis of testosterone. If it influenced a reaction which followed the synthesis of 17α-hydroxyprogesterone, either a decline or no change in the rate of its liberation would be anticipated.

For the moment, this suggestion that the excretion of pregnanetriol of testicular origin is associated with the biosynthesis and secretion of androgen by interstitial cells appears to be the most likely explanation for the phenomenon. It may, however, have a different meaning. The enhanced excretion of pregnanetriol could be related to the secretion of a nonandrogenic testicular product, *i.e.*, estrogen (22). In connection with any consideration that cells other than the interstitial cells of Leydig could be the source of the pregnanetriol precursor, it should be noted that in Subject E.B. (Table 1), the patient with almost complete absence of germ cells, urinary pregnanetriol rose sharply following administration of chorionic gonadotropin.

From these studies it seems clear that a portion of the urinary pregnanetriol in normal men may be derived from the testes.

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EFFECT OF HUMAN CHORIONIC GONADOTROPIN ON PLASMA CONCENTRATIONS OF 17-HYDROXY-CORTICOSTEROIDS, DEHYDROEPIANDRO-STERONE AND ANDROSTERONE IN MAN*

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ABSTRACT

Following the administration of chorionic gonadotropin to normal human males, a significant increase in the concentration of plasma androsterone was demonstrated. This increase was not observed in 5 normal young females. After administration of chorionic gonadotropin the diurnal variation in the concentration of plasma dehydroepiandrosterone was nearly obliterated in normal male and female subjects. Minor variations in plasma 17-hydroxycorticosteroid levels were found in both sexes.

It is suggested that the administration of chorionic gonadotropin with the subsequent measurement of plasma androsterone concentration may serve as a clinical test of testicular capacity in man.

ALTHOUGH a number of adequate chemical tests have been devised for the estimation of adrenocortical functions in man (1, 2), no satisfactory chemical test has been described for the evaluation of testicular function.

Savard *et al.* (3) were able to isolate testosterone from the perfusates of human testes and demonstrated, furthermore, that Δ^4-androstenedione-3,17 is a secretory product of human testicular tissue. Testosterone has been found in the spermatic vein blood of patients with carcinoma of the prostate (4). Brinck-Johnsen and Eik-Nes (5) observed that the concentrations of testosterone and Δ^4-androstenedione-3,17 in spermatic vein blood from the dog increased following the administration of human chorionic gonadotropin (HCG). Finally, the conversion of deuterium-labeled testosterone to deuterium-labeled androsterone has been reported in man (6) and androsterone has been isolated from human peripheral plasma (7).

If human chorionic gonadotropin exerts the same effect on the human testis as on the canine testis, it would be logical to assume that, following

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the administration of chorionic gonadotropin in man, the level of plasma androsterone should change. In this communication we wish to report plasma levels of 17-hydroxycorticosteroids (17-OHCS), dehydroepiandrosterone (DHEA) and androsterone in normal young human subjects following the administration of chorionic gonadotropin.

MATERIAL AND METHODS

The investigation was performed on 5 normal young women and 5 normal young men. Control blood plasma was obtained at 9 A.M., and a dose of 1000 units of human chorionic gonadotropin[1] (HCG) was administered intramuscularly. A 25-ml. specimen of blood was obtained at 10 A.M., 11 A.M., 12 noon, 1 P.M., 3 P.M. and 5 P.M. Heparin was used as an anticoagulant and the plasma was separated from the cellular layer immediately after the blood sample was withdrawn.

The plasma was extracted first with methylene chloride. 17-OHCS were determined in this extract by the method of Eik-Nes (8). The aqueous phase was then subjected to acid hydrolysis and DHEA was estimated in the hydrolysate (9). Plasma androsterone was detected by the following procedure: On the paper chromatogram used for the separation of plasma DHEA (9), the spots containing androsterone, as determined from the location of standard androsterone on a reference paper strip, were cut out and eluted with methanol. The dried eluate was dissolved in 10 ml. of benzene and extracted three times with 10 ml. of water each time. The Zimmermann procedure was then performed on the residue of the benzene solution. To establish the validity of the chemical methods used in this investigation for the determination of steroid hormones in blood plasma, a plasma pool of 90 ml. was divided into nine 10-ml. samples and assayed for 17-OCHS (8), DHEA (9) and androsterone. Mean[2] concentrations per 100 ml. of plasma were as follows: 18.1 μg. $\pm$0.3 (17.2–18.6) for 17-OHCS, 34.9 $\pm$0.7 (31.6–36.9) for DHEA and 17.3 $\pm$0.6 (14.6–19.8) for androsterone.

RESULTS

The plasma levels of 17-OHCS, DHEA and androsterone are presented in Table 1. Without the administration of HCG, DHEA and androsterone underwent a diurnal variation, as already reported (10). When HCG was given, an increase in the level of plasma androsterone occurred in each of the male subjects. The mean increase was significant from the second hour after HCG administration, whereas no effect of HCG on plasma androsterone concentration was observed in the women (Table 2).

Changes from the control diurnal variation of the plasma DHEA level were observed in both male and female subjects (Table 1). The changes consisted of a reduction in the magnitude of the usual decrease in level during the daytime hours. This did not become evident until three or four hours after administration of HCG. It should be noted that, following injection of HCG, the average change in plasma DHEA concentration was

[1] Supplied through the courtesy of the Upjohn Company, Kalamazoo, Michigan.

[2] Mean $\pm$ one standard error of the mean, and range.

TABLE 1. PLASMA CONCENTRATIONS OF DEHYDROEPIANDROSTERONE, ANDROSTERONE AND 17-HYDROXYCORTICOSTEROIDS IN NORMAL HUMAN SUBJECTS

Subject and sex	Condition	Dehydroepiandrosterone (μg./100 ml.)							Androsterone (μg./100 ml.)						
		9 A.M.	10 A.M.	11 A.M.	NOON	1 P.M.	3 P.M.	5 P.M.	9 A.M.	10 A.M.	11 A.M.	NOON	1 P.M.	3 P.M.	5 P.M.
1 (M)	Control	59.8	51.7	40.6	34.9	38.1	47.5	50.2	16.8	11.8	10.5	9.8	13.4	16.0	14.7
2 (M)		56.1	49.0	44.9	—	39.7	49.4	43.0	25.1	23.9	17.4	—	19.1	16.6	19.7
3 (M)		57.4	43.3	31.4	34.3	33.9	41.4	47.1	13.3	10.6	7.4	11.0	8.3	10.6	12.9
4 (M)		47.9	39.8	36.4	34.3	36.1	45.7	50.8	16.8	14.9	13.6	13.1	13.4	15.2	15.9
5 (F)		53.4	39.2	29.0	28.7	46.3	40.5	42.2	14.6	12.8	12.0	9.7	10.4	10.6	9.9
6 (F)		31.6	21.2	18.8	32.0	33.2	22.5	20.8	10.8	8.2	7.6	5.9	6.5	6.0	7.5
7 (F)		34.2	36.7	34.1	26.4	25.8	25.6	23.9	14.6	12.4	11.2	10.1	13.5	7.9	9.0
1 (M)	HCG at 9:10 A.M.	64.5	53.2	37.9	—	43.8	65.2	48.7	15.0	25.8	19.2	—	26.7	42.4	37.4
3 (M)		54.4	27.2	29.7	48.0	50.6	45.3	40.0	9.2	12.9	17.5	21.3	18.3	24.4	25.6
8 (M)		67.2	45.2	52.1	75.9	63.9	61.8	51.3	29.3	27.3	25.4	33.4	36.7	40.6	33.6
9 (M)		49.3	34.0	37.9	40.0	44.1	36.0	37.9	11.7	10.8	16.6	15.0	21.7	16.2	20.7
10 (M)		35.2	33.6	21.6	26.0	39.0	44.0	—	9.6	10.7	24.4	17.6	16.0	17.5	—
5 (F)		47.0	24.8	27.5	51.3	42.6	50.2	54.9	15.2	13.0	8.7	9.8	8.7	7.6	7.6
6 (F)		47.9	33.8	36.4	46.7	51.2	57.3	58.1	12.8	10.7	8.4	8.8	9.3	9.2	9.0
11 (F)		42.2	21.6	31.5	36.9	21.6	23.4	32.5	10.8	7.2	9.1	8.0	10.8	11.7	9.0
12 (F)		37.0	27.4	32.8	33.2	30.6	29.3	29.0	14.7	10.8	15.9	14.6	14.0	12.1	16.5
13 (F)		38.9	35.7	24.6	32.4	35.4	37.1	44.9	15.1	13.6	12.1	15.7	18.9	19.4	13.8

		17-Hydroxycorticosteroids (μg./100 ml.)						
		9 A.M.	10 A.M.	11 A.M.	NOON	1 P.M.	3 P.M.	5 P.M.
1 (M)	HCG at 9:10 A.M.	20.0	10.3	6.3	11.0	13.0	8.0	10.0
3 (M)		18.0	10.0	7.6	5.1	11.3	6.7	7.3
8 (M)		19.0	6.8	7.1	7.7	12.4	11.3	4.6
9 (M)		19.5	13.5	13.2	16.4	10.1	6.8	6.6
10 (M)		11.1	11.0	18.2	17.1	11.5	4.0	—
5 (F)		7.3	5.9	6.8	5.6	8.9	4.2	10.0
6 (F)		8.0	6.5	6.3	13.2	8.4	11.0	4.4
11 (F)		13.0	3.8	3.4	3.4	6.4	6.8	5.1
12 (F)		16.8	8.8	5.2	7.6	7.6	3.4	11.5
13 (F)		17.0	10.0	10.3	9.0	4.5	12.0	6.1

TABLE 2. PLASMA ANDROSTERONE: CHANGES FROM THE 9 A.M. CONCENTRATION, IN μG./100 ML. (MEAN CHANGES AND ONE STANDARD ERROR OF THE MEAN RECORDED)

	10 A.M.	11 A.M.	12 NOON	1 P.M.	3 P.M.	5 P.M.
Normal males (control)	−2.7±0.99	−5.8±0.94	−4.3±1.3	−4.4±1.2	−3.4±1.7	−2.2±1.3
Normal males (HCG-treated)	+2.5±2.3	+5.9±3.0	+9.5±2.3	+8.9±1.0	+13.3±3.8	+13.2±3.8
Treated/nontreated (probability)	<0.10>0.05	<0.02>0.01	<0.01	<0.01	<0.02>0.01	<0.01
Normal females (control)	−2.2±2.3	−3.1±0.34	−4.8±0.19	−3.2±0.48	−5.2±0.36	−4.5±0.32
Normal females (HCG-treated)	−2.7±0.47	−2.9±1.0	−2.3±1.2	−1.4±1.7	−1.8±2.0	−2.0±1.7
Treated/nontreated (probability)	<0.50	<0.50	<0.50>0.10	<0.50>0.10	<0.50>0.10	<0.50>0.10

never sufficient to reverse the usual diurnal fall, in contrast to the effect of this treatment on androsterone.

Plasma 17-OHCS levels were relatively unaffected by administration of HCG (Table 1). The average values at each time-period were significantly lower than the 9 A.M. value, as is characteristic of the normal pattern (11). However, inspection of the values for individual subjects shows that in each instance a secondary increase was observed between 11 A.M. and 3 P.M., in contrast to the smoothly decreasing curves which characterize the normal course of events. In 3 out of the 10 curves, one of these values exceeded the 9 A.M. value, a rare observation in the control curves. Thus minor and transient, but possibly significant, increases in the level of plasma 17-OHCS were observed.

DISCUSSION

Subsequent to the administration of chorionic gonadotropin, an increase in the concentration of plasma androsterone has been demonstrated in the normal human male. Chorionic gonadotropin will increase the secretion of testosterone and Δ^4-androstenedione-3,17 in the dog (5). The conversion of testosterone to androsterone has been demonstrated in man (6), and the metabolism of Δ^4-androstenedione-3,17 to androsterone in this species seems probable (12). Hence, we postulate that the observed increase in the plasma androsterone level following administration of chorionic gonadotropin to the normal male is the result of an increased secretion of testosterone and/or Δ^4-androstenedione-3,17 by the testis.

The marked normal diurnal fall in plasma DHEA concentration was nearly obliterated by administration of HCG. In this small series the changes were of questionable statistical significance. 17α-Hydroxypregnenolone has been found in testicular tissue (13) and has been isolated from the adrenal vein blood of dogs given ACTH (unpublished 1959 data of

Carstensen, H., Oertel, G. W. and Eik-Nes, K.). Further, we have observed the conversion of 17α-hydroxypregnenolone to plasma DHEA in the dog (14). It is possible that chorionic gonadotropin releases 17α-hydroxypregnenolone from testicular or adrenal tissue and makes this steroid available for metabolism to DHEA in man. Our data suggest, but do not prove, this possibility (15).

Administration of HCG increased the level of plasma 17-OHCS irregularly, regardless of sex. That HCG may affect the urinary excretion of 17-OHCS in man had already been reported (16). If ACTH-like activity were present in significant amounts in the HCG preparation used, a prompt rise in the plasma 17-OHCS level should have been observed regularly (17). Such was distinctly not the situation, as only Subject 10 showed this type of response. It is more difficult to be certain that the slightly painful injection of HCG did not produce these minor changes by indirect stimulation of endogenous ACTH release—the so-called "stress reaction." However, if this factor can account for the changes of plasma 17-OHCS concentration, these changes are unusual in their late appearance and irregular character as compared to the response to surgery and other events which have been demonstrated to affect the normal daytime fall in the concentration of plasma 17-OHCS significantly (18).

Since the level of plasma androsterone was not influenced by chorionic gonadotropin in the normal woman but was distinctly increased in the normal male, the measurement of plasma androsterone following administration of chorionic gonadotropin can probably be used to evaluate testicular capacity. This theory is at the present time being tested in our laboratory by measuring plasma androsterone levels in male subjects suffering from gonadal disorders.

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IN VIVO DISTRIBUTION OF SOME 17-HYDROXY-CORTICOSTEROIDS BETWEEN THE PLASMA AND RED BLOOD CELLS OF MAN*

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ABSTRACT

Various corticosteroids (cortisol, tetrahydrocortisol or tetrahydrocortisone intravenously, 1 mg. per Kg. of body weight; 4-C^{14}-cortisol intravenously, 1 microcurie; cortisol or cortisone acetate orally, 100 mg.) were administered to normal adult subjects. Blood samples were obtained at specific intervals following administration of steroid. 17-Hydroxycorticosteroid (17-OH-CS) extracts of red cells and plasma were prepared. In all cases, a significant fraction of the free 17-OH-CS of the various blood samples was associated with the red cells (average, 25.1 per cent; range, 17.7 to 36.5 per cent), whereas the total amount of the 17-OH-CS glucuronides was found in the plasma.

THE role played by the binding of steroids to plasma proteins in the transport of these hormones has been studied in some detail and this question has been reviewed by Sandberg *et al.* (1). Much less attention has been focused on the possible role of red blood cells in the transportation of steroids.

As far as cortisol is concerned, it has been reported that less than 10 per cent of the 17-hydroxycorticosteroids measured in blood were associated with the cells, but when blood was allowed to stand the steroids penetrated the red cells gradually (2). However, Peterson *et al.* (3) observed that 20 to 25 per cent of the 4-C^{14}-cortisol in a given volume of whole blood was associated with the red cells, as calculated by the difference between counts of radioactivity on whole blood and on plasma. The same authors found that

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neither the time of incubation (one or two hours) nor the temperature (25° or 37° C.) altered the distribution. Similar data have been reported by others (4, 5). In addition, Wu and Mason (5) observed that during the first 48-hour period following collection of blood, the corticosteroid levels of the plasma and of the red cells did not vary significantly.

The purpose of the present work was to study further the distribution of cortisol between plasma and red cells and to extend this work to other corticosteroids known to be metabolites of cortisol, such as cortisone, tetrahydrocortisone and tetrahydrocortisol.

METHODS

Subjects. Eight normal persons, 4 males and 4 females, 23 to 33 years of age, were the subjects of this study.

Administration of corticosteroid and collection of blood samples. Subjects 1, 2 and 3 received an intravenous infusion of cortisol[1] (1 mg. per kilogram of body weight) over a 30-minute period. Subjects 4, 5 and 6 were given an oral dose of 100 mg. of cortisol,[1] and on another occasion several months later were given an oral dose of 100 mg. of cortisone acetate.[2] One microcurie of 4-C^{14}-cortisol[3] was administered intravenously to Subject 7, according to techniques previously described (6). In another study, Subjects 1, 2, 4 and 8 received tetrahydrocortisol or tetrahydrocortisone[2] intravenously (1 mg. per kilogram of body weight) over a 30-minute period.

Blood samples were collected prior to, and at specific intervals following administration of steroid as indicated in Tables 1 and 2.

Microhematocrits. Duplicate microhematocrits were run on each blood sample, using an International micro-capillary centrifuge (Model 490) and heparinized capillary tubes of 75 mm. in length and 1.0 mm. in diameter. The tubes were centrifuged at 11,000 r.p.m. for five minutes and the percentage of red cell volume was determined by a micro-capillary reader.

Centrifugation of the blood samples. An International centrifuge, size 2, model V with a No. 240 head and 100-ml. cups giving a rotating radius at the tip of the cups of 25 cm., was used. All samples were centrifuged at 2,000 r.p.m. for thirty minutes; the relative centrifugal force was approximately 1,100 times gravity.

Preparation of extracts of free 17-hydroxycorticosteroids. A volume of 100 per cent ethanol equal to three times that of the plasma was added to the plasma samples. The

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[1] Cortef, a preparation of cortisol in 50 per cent ethanol (5 mg. of free steroid per 1 ml.) was used. When given intravenously, the proper amount of Cortef was added to 200–300 ml. of 0.9 per cent NaCl solution and the mixture was administered over a 30-minute period. Cortef was diluted with approximately 200 ml. of tap water, when given orally. Cortef was graciously supplied by Dr. C. J. O'Donovan of the Upjohn Company, Kalamazoo, Michigan.

[2] Cortisone acetate and tetrahydrocortisone were made available through the kindness of Dr. Augustus Gibson of Merck Sharp & Dohme, Rahway, New Jersey.

[3] The 4-C^{14}-cortisol was generously provided by the Endocrinology Study Section, Division of Research Grants, National Institutes of Health, Bethesda, Maryland. Its specific activity was 1.467 microcuries per micromole.

mixture was centrifuged and the supernatants were decanted. The residue was washed twice with ethanol, using an amount equal to the initial plasma volume. After centrifugation, the supernatants were added to those of the first extraction and the pooled extracts were evaporated to dryness. The residue was dissolved in a volume of distilled water approximately equal to that of the initial plasma samples. This aqueous preparation was extracted three times with twice its volume of chloroform. The chloroform extracts were treated according to the method described by Nelson and Samuels (7) as modified by Eik-Nes *et al.* (2).

The blood cells were kept overnight in a freezer and then extracted with ethanol;

TABLE 1. EXAMPLE OF THE CORRECTION APPLIED TO THE RESULTS OBTAINED FOR THE AMOUNT OF 17-OH-CS ASSOCIATED WITH RED BLOOD CELLS, IN SUBJECT 1, WHO RECEIVED AN INTRAVENOUS INFUSION OF CORTISOL OVER A 30-MINUTE PERIOD

Time after infusion of F	Blood vol. (ml.)	Micro-hemat.	Obtained plasma vol.	Expected plasma vol.	17-OH-CS (μg.)						
					Total blood	Plasma			Red cells		
						Gross	Cor-rected	% Total	Gross	Cor-rected	% Total
Free 17-OH-CS											
Control	46	42	25.1	26.7	3.64	2.63	2.80	77.0	1.01	0.84	23.0
1 hr.	30	42	16.5	17.4	22.37	14.70	15.50	69.3	7.67	6.87	30.7
2 hrs.	31	43	15.5	17.7	13.73	7.75	8.85	64.4	5.98	4.88	35.6
4 hrs.	30	42	16.1	17.4	5.63	3.34	3.61	64.1	2.29	2.02	35.9
6 hrs.	29	43	15.6	16.5	2.17	1.53	1.62	74.7	0.64	0.55	25.3
17-OH-CS liberated by β-glucuronidase hydrolysis											
Control	46	42	25.1	26.7	1.15	1.15	1.22	106.0	0	0	0
1 hr.	30	42	16.5	17.4	5.38	5.15	5.43	101.0	0.23	0	0
2 hrs.	31	43	15.5	17.7	11.30	9.76	10.95	97.0	1.54	0.45	3.0
4 hrs.	30	42	16.1	17.4	10.77	10.46	11.32	105.0	0.31	0	0
6 hrs.	29	43	15.6	16.5	8.55	8.16	8.64	101.0	0.39	0	0

the dried residue was dissolved in water and this aqueous preparation was treated as described for the plasma samples. Following chromatography with Florisil, the extracts of plasma with gross impurities, and all the extracts of red cells were submitted to fractionation with benzene, water and chloroform carried out as follows:

The residue was dissolved in 2.5 ml. of distilled benzene and this was extracted once with 5 ml. and once with 10 ml. of water; then the water was extracted three times with 30 ml. of chloroform, which was evaporated. This dry residue constituted the final extract.[4] The latter step in the technique is similar to the fractionation with benzene, water and chloroform described by Talbot *et al.* (8) and used by Eik-Nes (9).

Preparation of extracts of the 17-hydroxycorticosteroids conjugated with glucuronic acid.

[4] When the fractionation of red-cell extracts with benzene, water and chloroform was not carried out, the absorption spectrum of the chromogen resulting from the Porter-Silber reaction was unspecific and did not present the characteristic maximum absorption at 410 mμ.

TABLE 2. DISTRIBUTION BETWEEN PLASMA AND RED BLOOD CELLS OF THE FREE 17-OH-CS AND OF THE 17-OH-CS LIBERATED BY β-GLUCURONIDASE HYDROLYSIS

Subjects	Steroid administered	Time after steroid administration	Per cent of total blood steroid associated with red blood cells	
			Free	Liberated by β-glucuronidase
#2, B.L., ♀	Cortisol, i.v., 1 mg./Kg. body wt.	Control	19.2	1.8
		1 hr.	33.3	0
		4 hrs.	22.8	0
		6 hrs.	18.9	1.2
#3, J.H., ♂	Cortisol, i.v., 1 mg./Kg. body wt.	Control	17.7	0
		1 hr.	34.2	0
		2 hrs.	30.1	0
		4 hrs.	27.8	1.0
		6 hrs.	32.3	1.2
#7, J.F., ♀	4-C^{14}-cortisol, i.v., 1 μc.	20 mins.	27.0	0
		60 mins.	18.2	0
		120 mins.	20.5	2.0
		240 mins.	26.8	0
		360 mins.	21.5	0
#4, C.M., ♂	Cortisol, orally, 100 mg.	60 mins.	26.0	0
		120 mins.	25.4	0
#3, J.H., ♂	Cortisol, orally, 100 mg.	60 mins.	28.0	0
		120 mins.	30.5	0.3
#6, R.B., ♂	Cortisol, orally, 100 mg.	60 mins.	27.5	0
		120 mins.	22.6	0
#4, C.M., ♂	Cortisone acetate, orally, 100 mg.	60 mins.	19.8	0
		120 mins.	21.6	0
#5, J.B., ♂	Cortisone acetate, orally, 100 mg.	60 mins.	20.1	0
		120 mins.	24.7	0
#1, P.W., ♀	Tetrahydrocortisol, i.v., 1 mg./Kg. body wt.	Control	32.5	0
		40 mins.	23.2	0
		70 mins.	20.0	0.3
		100 mins.	20.0	0.4
#2, B.L., ♀	Tetrahydrocortisone, i.v., 1 mg./Kg. body wt.	46 mins.	27.0	0.9
		74 mins.	30.8	0
		100 mins.	36.5	0.2
#4, C.M., ♂	Tetrahydrocortisone, i.v., 1 mg./Kg. body wt.	40 mins.	18.0	1.1
		70 mins.	17.7	0.5
		100 mins.	24.2	0
#8, E.L., ♀	Tetrahydrocortisone, i.v., 1 mg./Kg. body wt.	40 mins.	21.1	0.6
		70 mins.	19.3	0
		100 mins.	18.2	0

Following removal of the free 17-hydroxycorticosteroids (17-OH-CS), β-glucuronidase (600–700 units per ml. of plasma) and 2 *M* acetate buffer were added to the aqueous preparations of plasma and red cells. The pH was adjusted to 4.7 ± 0.05. After an incubation period of fifteen hours at 47° C., the 17-OH-CS freed by hydrolysis were extracted with chloroform and evaporated to dryness. The residue was applied onto a column of 3 Gm. of silica gel, using 20 ml. of Skellysolve C:ethyl acetate (3:1) and 10 ml. of ethyl acetate:methanol (4:1). Only the second fraction was saved, evaporated to dryness, and the residue applied onto a Florisil column, as described by Nelson and Samuels (7). The 25 per cent methanol-in-chloroform fraction was collected and evaporated to dryness. The residue was dissolved in 2.5 ml. of distilled benzene for fractionation with benzene, water and chloroform.

When one of the 3 steps of the purification of the extracts (*i.e.*, chromatography on a column of silica gel, chromatography on a column of Florisil, and fractionation with benzene, water and chloroform) was omitted, the chromogens resulting from the Porter-Silber reaction did not show a maximum absorption at 410 mμ. The recovery of 5 μg. of tetrahydrocortisone through the entire procedure was approximately 75 per cent. The results were corrected for the recovery obtained for each series of determinations.

Quantitative determination of 17-hydroxycorticosteroids. The 17-OH-CS content of the various extracts was determined by the reaction of Porter and Silber (10).

The radioactivity of extracts of the plasma and blood cells from Subject 7 was determined as previously described (6).

Correction for the plasma remaining with the blood cells after centrifugation. Following centrifugation of the various samples of blood, the plasma was removed as completely as possible and measured carefully. Knowing the total blood volume of each sample and its microhematocrit, the expected plasma volume was calculated. This value was compared with the plasma volume obtained and the difference between the two values was considered to represent the amount of plasma remaining with the red cells.

The 17-OH-CS content of the plasma remaining with the red cells of the various samples was calculated. This was subtracted from the total 17-OH-CS content of the red cells, to obtain the corrected red cell value. An example of the correction is shown in Table 1.

RESULTS

Figure 1 shows the concentrations in plasma of free 17-OH-CS and of 17-OH-CS liberated by β-glucuronidase hydrolysis in subjects who received an intravenous infusion of cortisol (1 mg. per Kg. of body weight), and Figure 2 shows the results obtained following the intravenous infusion of tetrahydrocortisone.

The "corrected" concentrations of 17-OH-CS in the red cells have been expressed as a percentage of the amount of 17-OH-CS in the samples of whole blood. From 17.7 to 36.5 per cent of the total free 17-OH-CS of the various blood samples was associated with the red blood cells, but no significant amounts of glucuro-conjugates appeared to be associated with the red cells (Tables 1 and 2). The results did not depend upon the nature of the corticosteroid administered or its mode of administration.

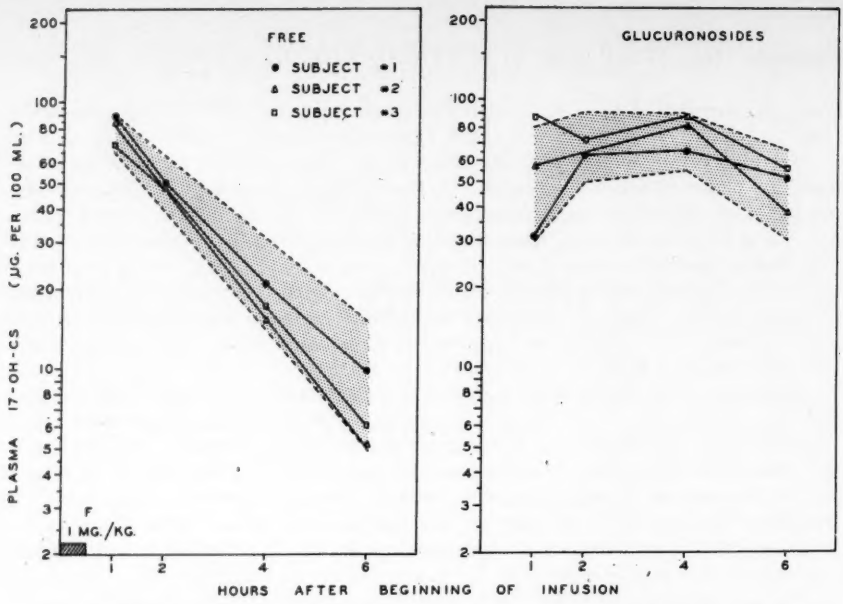

FIG. 1. Plasma levels of 17-OH-CS following intravenous administration of cortisol (1 mg./Kg. body weight) over a 30-minute period to Subjects 1, 2 and 3. The shaded area shows the extreme range of variation obtained in 15 normal adult subjects; the dotted lines depict the upper and lower limits of the range.

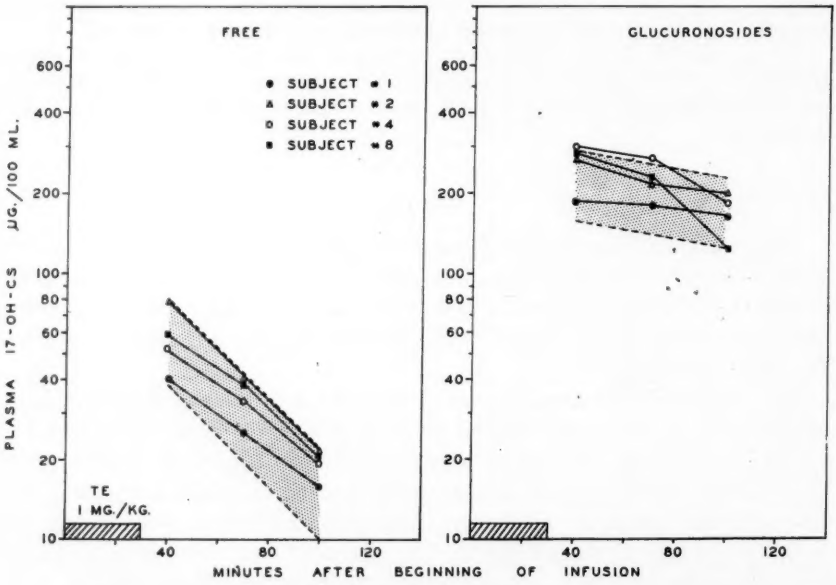

FIG. 2. Plasma levels of 17-OH-CS following intravenous administration of tetrahydrocortisone or tetrahydrocortisol (1 mg./Kg. body weight) over a 30-minute period to Subjects 1, 2, 4 and 8. The shaded area shows the extreme range of variation obtained in 10 normal adult subjects; the dotted lines depict the upper and lower limits of the range.

DISCUSSION

The calculation of the distribution of 17-OH-CS between plasma and red cells of a given sample of blood is based on the assumption that the microhematocrit reading denotes the real volume of the erythrocytes. This assumption was found to be valid (11) although, theoretically, the percentage of 17-OH-CS associated with the red cells it still slightly overestimated due to the fact that minimal amounts of plasma are contained in the volume of red cells.

In one *in vivo* study, Peterson *et al.* (3) found that 20 to 25 per cent of the radiocortisol in whole blood was associated with the red cells. The method used by these authors for the calculation of the distribution was somewhat similar to that used in the present paper. Wu and Mason (5) found that in 4 samples of blood the concentration of Porter-Silber chromogens in the erythrocytes was 15 to 33.6 per cent of the concentration in the plasma, but the report did not mention whether a correction had been applied for the plasma trapped between the red cells following standard centrifugation. In the present study an average of 25.1 per cent of the free 17-OH-CS in whole blood was observed to be associated with the red cells. It is of interest that similar results were obtained following administration of either large amounts of nonisotopic cortisol or microamounts of 4-C^{14}-cortisol. In addition, the values were in the same range for either cortisol, cortisone, tetrahydrocortisone or tetrahydrocortisol.

Some of the extracts of plasma and of red cells were chromatographed on paper, using Bush's system (12) benzene:methanol:water (100:55:45), and the papergrams were colored with blue tetrazolium reagent. It was found that, following infusion of tetrahydrocortisone, the material measured as free Porter-Silber chromogen was tetrahydrocortisone itself in both plasma and red-cell extracts. Following infusion of cortisol, the plasma extracts contained cortisol, tetrahydrocortisone and tetrahydrocortisol in the approximate proportion of 55, 25 and 20 per cent of the total, respectively. The proportion of these three 17-OH-CS was similar in the extracts of red cells, and the distribution of cortisol based on the measurement of 17-OH-CS rather than specifically on the measurement of cortisol is acceptable.

Peterson *et al.* showed that the *in vitro* distribution of cortisol between plasma and red cells was similar to that observed *in vivo*, and that one or two hours of incubation at either 25° or 37° C. had no influence on the results (3). We have made similar observations; in addition, it was found that the distribution was immediate (13). It was also found that the distribution coefficient of cortisol was about 2.90 in the system plasma:red cells and 0.44 in the system saline:red cells (13). In our previous *in vivo* work

(6) with 4-C^{14}-cortisol very little activity had been detected in the red cell fraction; since we had washed the red cells twice, with twice their volume of saline, we had been removing most of the activity accompanying the red cells. Sandberg *et al.* (1) also washed the erythrocytes three times with saline, and consequently their distribution studies are not valid. However, the error is much smaller for steroids like estrone, which has a distribution coefficient in the system saline:red cells of only 0.094 to 0.125 (11).

In vivo studies with 16-C^{14}-estrone and estradiol have shown that a significant amount of radioactivity was associated with the erythrocytes of the blood samples collected at various times after administration of the steroids (14, 15). Only 10 to 20 per cent of the total activity detected in the red cells was readily extractable by chloroform; this suggests that a large fraction of the total activity was in a conjugated form. However, β-glucuronidase hydrolysis freed only a small part of the activity remaining after removal of the free fraction. In the present study with corticosteroids, no 17-OH-CS glucuronides were found to be associated with the red cells, but the total conjugated 17-OH-CS fration was not studied, and consequently we cannot exclude the possibility that 17-OH-CS conjugates other than glucuronide conjugates are associated with the red cells.

Based on the findings of this and other studies (3–5), the red blood cells appear to play a role in transport of cortisol which is not negligible. However, under normal circumstances, the distribution of free cortisol, cortisone, tetrahydrocortisone and tetrahydrocortisol between plasma and red cells is constant within a wide range of concentrations.

The data presently available do not permit a decision as to whether free corticosteroids are bound on the surface of the red cells or whether they penetrate the cells. Previous studies with estrone suggested that this steroid is adsorbed on the surface of the cell (11).

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VARIATIONS IN ENDOCRINE GLAND FUNCTION IN POSTPARTUM PITUITARY NECROSIS*

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ABSTRACT

Postpartum pituitary necrosis in 4 patients was manifest by classic panhypopituitarism with secondary insufficiency of the thyroid, adrenal cortex, and ovary. Three other patients presented atypical syndromes characterized by virtually normal thyroid function, variable degrees of adrenocortical insufficiency, and normal to slightly elevated urinary excretion of gonadotropin, with or without the resumption of menses. Low 24-hour thyroidal I^{131} uptakes were associated with low urinary gonadotropin levels, and normal uptakes with higher gonadotropin levels. It is inferred that in some cases of postpartum pituitary necrosis there may be decreased ACTH secretion, nearly normal TSH secretion, and restored gonadotropin secretion which, however, may not be adequate for normal ovarian function.

ABOUT twenty years ago Sheehan described the natural history, symptoms, signs and pathologic findings of the syndrome which follows portpartum necrosis of the adenohypophysis (1). Correlation of clinical data with autopsy material showed that excessive hemorrhage and shock during delivery was followed by a variable degree of necrosis of the adenohypophysis. The severity of the ensuing syndrome corresponded with the extent of the pituitary destruction, symptoms being slight with an estimated 60 per cent necrosis, moderate with 75 per cent, and severe with 95 per cent. The clinical details of the severe syndrome of panhypopituitarism were emphasized, although examples of lesser grades of hypopituitarism were thought to be more common.

Subsequently, some aspects of the less severe syndromes have been described, and the variable symptomatology stressed. There may be a marked dissociation in the degree of the functional insufficiency of the thyroid, adrenal cortices, and gonads (2, 3). This variable impairment of tar-

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get-gland functions, and even of various adrenocortical functions within the same patient, may occur also in hypopituitarism resulting from pituitary tumors (4).

The histories, physical findings, and endocrine functions of 7 females who presumably had postpartum necrosis of the adenohypophysis are presented in some detail. All patients had abnormal deliveries, and dated the onset of their symptoms therefrom. Six of them are known to have suffered from excessive hemorrhage or shock following delivery. The cases collectively illustrate wide variations of thyroid, adrenocortical and ovarian functions, as previously described (1–4). Of particular interest is the fact that in 3 of the patients, thyroid function was normal and urinary gonadotropin was detected, although there was impairment of adrenocortical function. The detailed case histories which are presented here emphasize the variability of the manifestations of postpartum pituitary necrosis and may prove useful in the diagnosis of other cases of this syndrome.

METHODS

Conventional techniques were used for determination of 24-hour thyroidal radioiodine (I^{131}) uptake (normal, 10 to 40 per cent) and for the level of serum protein-bound iodine (PBI, normal, 4 to 8 μg. per 100 ml.). The 24-hour excretion of urinary neutral 17-ketosteroids (17-KS, normal for adult women, 6 to 14 mg.) was determined by the method of Klendshoj, Feldstein and Sprague (5), and of urinary 17-hydroxycorticosteroids (17-OHCS, normal, 2.5 to 8 mg.) by the Cranny modification of the method of Porter and Silber (6). The concentration of blood glucose (normal fasting values, 65 to 110 mg. per 100 ml.) was determined by the Nelson modification of the method of Somogyi (7). Urinary gonadotropin (normal values for adults, greater than 6.5 but less than 26 mouse uterine units (m.u.u.) per twenty-four hours) was assayed by the method of Klinefelter, Albright and Griswold (8). Serum magnesium concentration (normal, 1.4 to 2.2 mEq. per liter) was measured by a modification of the method of Kunkel, Pearson and Schweigert (9).

Whenever the initial 24-hour thyroidal I^{131} uptake was less than normal, a single intramuscular dose of 10 units of thyroid-stimulating hormone (TSH) (Thyrotropar, Armour) was given intramuscularly simultaneously with the repeat tracer dose of I^{131}, or a dose of 10 units was administered daily for four days and the repeat tracer dose of I^{131} was given on the fourth day. Urinary 17-KS, and in several cases 17-OCHS, were studied on four consecutive days. During the last two days, an eight-hour infusion containing 25 units of adrenocorticotropin (ACTH) was given intravenously (10). Other studies of adrenal function were the Robinson-Kepler-Power (RKP) water test (11), and the insulin tolerance test using 0.1 unit of unmodified insulin per Kg. of body weight (12).

CASE PRESENTATIONS

The cases are presented approximately in order of decreasing severity. The relevant laboratory data obtained during the initial examination of each patient are presented in Table 1. The results of subsequent examinations are presented in the text.

TABLE 1. LABORATORY DATA OBTAINED DURING INITIAL EXAMINATIONS

	Case 1 SF	Case 2 DL	Case 3 BS	Case 4 CD	Case 5 GA	Case 6 BC	Case 7 TL
Hemoglobin (Gm./100 ml.)	9.6	9.4	10.3	9.8	6.9	13.6	12.6
Blood urea nitrogen (mg./100 ml.)	22	30	28	18	12		17
Serum Na (mEq./L)	140.0	138.0	137.8	134.4	142.8	140.0	141.6
Serum K (mEq./L)	4.6	5.2	4.9	4.9	3.6	4.2	5.1
Serum CO_2 (mEq./L)	25.4	26.2	29.8	29.4	19.3	28.8	24.0
Serum Cl (mEq./L)	97.0	105.0	95.0	103.0	103.9	97.0	103.0
Serum Ca (mg./100 ml.)	10.0	10.6		8.2	10.1	9.0	
Serum PO_4 (mg./100 ml.)	4.2	4.9		5.0	3.0	5.3	
Serum Mg (mEq./L)		1.7		1.4		1.9	
Thyroidal 24-hr. I^{131} uptake (% dose)							
Control	0.0	4.7*	1.6	4.1	31.9	19.6	23.5
After TSH, 10 u. once			10.5				
After TSH, 10 u. q.d. for 4 days	0.4	24.4*					
Serum PBI (μg./100 ml.)	2.3	20.0*	1.7	2.0	4.5	3.1	4.3
Serum total cholesterol (mg./100 ml.)	296	424	278	234	136	463	181
Urinary 17-KS (mg./24 hrs.)							
Day 1. Control	0.7	0.9	0.8	1.3	0.7	4.1	3.2
Day 2. Control	1.1	1.2	0.6	2.5	0.5	3.2	2.1
Day 3. ACTH, 25 u.	0.6	1.4	1.5	2.8	1.8	6.4	4.9
Day 4. ACTH, 25 u.	1.3	2.0	2.8	3.5	3.6	11.5	5.3
Urinary 17-OHCS (mg./24 hrs.)							
Day 1. Control			1.7		0.5	1.0	
Day 2. Control			0.6		0.6	0.7	
Day 3. ACTH, 25 u.			5.5		3.0	7.7	
Day 4. ACTH, 25 u.			10.2		5.6	12.9	
RKP water test							
9-hour night vol. (ml.)	100	250	260	400		360	230
Largest hour vol. (ml.)	30	40	36	50		440	120
Fasting blood glucose, and/or insulin tolerance test levels (mg./100 ml.)	75	70	65	64	88	73	60
20 mins. after insulin		38		25		19	18
30 mins. after insulin		24		15		16	27
45 mins. after insulin		28		†		23	34
60 mins. after insulin		28				28	41
90 mins. after insulin		23				28	41
120 mins. after insulin		28				24	55
Urinary gonadotropin (m.u.u./24 hrs.); number of assays at each level							
<6.5	Two	Two	Two	Three	Two		
>6.5, <13			Two			Two	
>13, <52						Two	Three
>52, <104							Three

* Exogenous iodide from cholecystography.

† Discontinued because of severe hypoglycemic reaction.

Case 1

S.F. (U.H. 246-678), a 57-year-old white female, was admitted in 1956 because of progressive weakness. Lactation followed her first 2 deliveries. The third labor at age 21 was complicated by profuse hemorrhage, but no blood transfusions were given. Lactation did not occur, and permanent amenorrhea ensued. During the next ten years

axillary, pubic and scalp hair was gradually lost. Weakness and malaise became incapacitating; during the last five years she was continually bedridden.

The patient appeared about fifteen years older than her stated age. Her weight was 44 Kg., height 152 cm., and blood pressure 84/52 mm. Hg. The scalp was nearly bald. The skin was pale, dry and scaly. On the face were multiple lesions shown by biopsy to be basal-cell carcinomas, squamous-cell carcinomas and benign keratoses. Ophthalmoscopic findings and visual fields were normal. The gums were edentulous. The thyroid gland was not palpable. The mammary tissue seemed atrophic, and the nipples and areolae were markedly depigmented. Axillary and pubic hair was absent. The vaginal mucosa was smooth, pale and atrophic. The cervix was only a dimple, and the uterus and ovaries were not palpable.

Roentgenograms of the skull and chest were unremarkable. The electrocardiogram showed low voltage, partial A-V block, and nonspecific primary T-wave changes.

Cortisone acetate was given in a dosage of 25 mg. daily; later desiccated thyroid was added and increased gradually to 120 mg. daily. The clinical improvement thereafter was striking. The patient became physically and mentally active. Scalp hair regrew, although axillary and pubic hair remained absent. The electrocardiogram became normal. Two urinary gonadotropin assays, eight months and fourteen months after beginning endocrine therapy, showed less than 6.5 m.u.u. per twenty-four hours.

Comments. Failure of the thyroid gland to concentrate I^{131} and the low level of serum PBI indicated hypothyroidism. After 10 units of intramuscular TSH daily for four days the 24-hour thyroidal I^{131} uptake was not increased. This result usually denotes primary hypothyroidism. However, the history, the total loss of body hair, the normal serum cholesterol level, and the absence of urinary gonadotropin in a postmenopausal female indicated hypothyroidism of pituitary origin. Furthermore, it has been noted that in 15 of 60 patients with hypopituitarism due to various causes the thyroid gland was only a small mass of dense scar tissue; this atrophy was as severe as in primary hypothyroidism (13). Thyroid glands showing such severe atrophy probably will not respond to TSH. This hypothesis finds some confirmation in a recent observation that 3 of 11 cases of secondary hypothyroidism were completely refractory to TSH (14).

Adrenocortical insufficiency was indicated by the extremely low urinary excretion of 17-KS on the two control days. During the following two days of stimulation with intravenous ACTH, urinary 17-KS did not increase. In adrenocortical insufficiency secondary to hypopituitarism the adrenal cortex responds gradually to ACTH, and three to five days of stimulation may be required for a significant rise in urinary steroidal excretion (10). The urinary 17-OHCS increment after administration of ACTH is larger and occurs more rapidly than the 17-KS increment, but 17-OHCS were not determined in this patient. It is probable that more prolonged stimulation with ACTH and determination of urinary 17-OHCS would have demonstrated adrenocortical responsiveness.

The onset of amenorrhea following parturition and the absence of uri-

nary gonadotropin in a postmenopausal female point to adenohypophyseal insufficiency as the cause of the extremely severe atrophy of the genital organs.

In summary, the laboratory data indicated hypothyroidism, adrenocortical insufficiency, and pituitary insufficiency with respect to gonadotropin. Stimulation of the thyroid gland and of the adrenal cortex with appropriate tropic hormones was not followed by demonstrably increased functioning of these two glands. Nevertheless, consideration of the limitations of the tests and correlation of the results with the history and physical findings indicate panhypopituitarism due to postpartum pituitary necrosis.

Case 2

D.L. (OMRH 682), a 52-year-old white female, was admitted in 1957. At age 19 she gave birth to her only child. High forceps were used and a blood transfusion was given. The patient was so critically ill that the presence or absence of lactation cannot be recalled. She did not nurse the baby. Permanent amenorrhea followed. From age 20 to 30 she noted progressive loss of body hair. Intolerance to cold developed, and she complained of syncope during hot weather. Dryness of the skin, marked weakness, and easy fatigue were noted. Perspiration occurred only on the face. During the six months prior to admission she had intermittent diarrhea without fever.

The patient appeared chronically ill, apathetic, and older that her stated age. Her weight was 43.8 Kg., height 146 cm., and blood pressure 180/90. The facies appeared moderately myxedematous, with puffiness of the eyelids and wrinkling of the skin. The skin was pale and lacked the normal capillary flush. The optic fundi and visual fields were normal. The gums were nearly edentulous and showed pyorrhea. The scalp hair was coarse and dry. There was no axillary sweating, and axillary and pubic hair was absent. The thyroid gland was not palpable. The nipples and areolae were depigmented. The vaginal mucosa was thin, pale and smooth. The cervix and body of the uterus were about half normal size. The ovaries were not palpable. Deep tendon reflexes showed a lag of the relaxation phase.

Skull roentgenograms were normal. Chest roentgenograms indicated slight enlargement of the left ventricle. The electrocardiogram showed low voltage, partial A-V block, and nonspecific primary T-wave changes.

Results of several urinalyses were normal. Intermittently, slight albuminuria was present; two 24-hour urine specimens contained 0.9 Gm. and 2.4 Gm. of protein. The serum was lactescent. Serum cholesterol and phospholipid concentrations were abnormally high, and analytical ultracentrifugation revealed abnormally large amounts of very low density lipoproteins. The total serum protein level, albumin/globulin ratio, bromsulfalein retention and phenolsulfonphthalein excretion were normal. Three basal metabolic rates were −39, −40, and −32 per cent.

A few months after the first admission she was readmitted with flank pain, fever, dysuria, and pyuria. Urinary cultures showed paracolon bacilli, which disappeared with antibiotic therapy. Fourteen months after the first admission, while taking cortisone acetate (25 mg.) and thyroid (60 mg.) daily, she was admitted to University Hospital in a stuporous condition following a nonspecific upper respiratory infection. Blood pressure was 180/60. Oral temperature was 104.6° F. She improved rapidly following intravenous hydrocortisone therapy.

Comment. The patient clinically was hypothyroid. A few days before admission she had received an iodinated compound in preparation for a cholecystogram, and the serum PBI level was spuriously high. The low 24-hour thyroidal I^{131} uptake may have been due to the exogenous iodine and/or to hypothyroidism. Following 10 units of intramuscular TSH daily for four days the 24-hour I^{131} uptake increased to normal. This result makes primary hypothyroidism unlikely. Hypercholesterolemia usually suggests primary hypothyroidism, but may occur secondarily to hypopituitarism (3, 15). In this patient the lactescence and the high level of very low density lipoproteins may be attributed to an excessive mobilization of lipid associated with severe undernutrition. The hyperlipemia disappeared and the hypercholesterolemia was reduced in association with improvement in her nutrition and a weight gain of 5 Kg. which occurred following several weeks of treatment with cortisone (but no thyroid) and an unrestricted diet.

The low urinary excretion of 17-KS on the two control days indicated hypofunction of the adrenal cortex. On the second day of ACTH stimulation the 17-KS showed an increment, but it was of doubtful significance. However, the marked loss of skin pigmentation and the normal serum electrolyte levels indicated secondary rather than primary adrenocortical insufficiency.

Amenorrhea since age 19, marked atrophy of the genital organs, and absence of urinary gonadotropin in a 57-year-old woman suggested that there had been decreased pituitary secretion of gonadotropin for many years.

Hypertension associated with hypopituitarism has been observed (2, 13). In this patient chronic pyelonephritis was considered to be the initial cause of the hypertension.

In summary, the clinical history and physical findings were characteristic of panhypopituitarism. The laboratory data excluded primary hypothyroidism and indicated adrenocortical insufficiency. The 17-KS response to ACTH did not differentiate primary from secondary adrenocortical insufficiency, but the former was reasonably excluded by other criteria.

Case 3

B.S. (OMRH 564), a 36-year-old para 5, gravida 5, white female, was admitted in 1956. Seven years earlier the last delivery had been complicated by a retained placenta and excessive blood loss. The patient lost consciousness and was given blood transfusions. During the next two weeks she had severe occipital headaches, nausea and vomiting. Lactation did not occur, though she had nursed her previous babies. Permanent amenorrhea ensued. Her general health deteriorated and she became very weak. Pubic hair did not regrow; scalp and axillary hair was gradually lost. She noted dryness of the skin, intolerance to cold, and decreased sweating.

This lethargic female appeared twenty years older than her stated age. Her weight was 57.8 Kg., height 156.6 cm., and blood pressure 80/60. The facies seemed hypothyroid. The skin was dry and waxy. The scalp hair was coarse, dry and sparse; axillary and

pubic hair was absent. Ophthalmoscopic findings and visual fields were normal. The thyroid gland was not palpable. The gingivae presented marked pyorrhea. The breasts did not seem atrophic; nipples and areolae were pale and depigmented. The vaginal mucosa was thin, pale, smooth and atrophic. The cervix was approximately one-third normal size, and the corpus uteri was tiny. The ovaries were palpable, but very small.

Roentgenograms of the skull and chest were unremarkable. The electrocardiogram showed low voltage and nonspecific primary T-wave changes.

The patient was seen fifteen months later. She had been taking cortisone acetate (25 mg.) and desiccated thyroid (180 mg.) daily. She was markedly improved. No menstruation had occurred. The electrocardiogram was normal. Urinary gonadotropin excretion was less than 6.5 m.u.u. per twenty-four hours.

Comment. The clinical impression of hypothyroidism was substantiated by the low serum PBI level and the low thyroidal I^{131} uptake. The increased I^{131} uptake after 10 units of TSH demonstrated the pituitary origin of the hypothyroidism.

Urinary 17-KS excretion on control days was low, and increased nearly four times during the second day of ACTH stimulation. The control urinary 17-OHCS excretion similarly was low, and the marked rise following administration of ACTH showed unequivocally the responsiveness of the adrenal cortex to ACTH.

The amenorrhea and atrophy of the pelvic organs indicated ovarian failure. On two occasions no urinary gonadotropin was demonstrable; on two other occasions the excretion was greater than 6.5, but less than 13 m.u.u. per twenty-four hours, which is normal in menstruating females, but less than expected in primary ovarian failure. Therefore, a quantitative or qualitative abnormality of pituitary gonadotropin secretion was presumably present.

In summary, the patient presented panhypopituitarism due to postpartum pituitary necrosis. The thyroid gland and adrenal cortex showed diminished functions, but they responded readily to their respective tropic hormones. Although ovarian failure was obvious, urinary gonadotropin was intermittently absent or at the lower limit of normal.

Case 4

C.D. (OMRH 699), a 30-year-old Negro female, was admitted in 1957. Her first two deliveries at age 22 and 23 were unremarkable, and lactation was normal. During labor of the third pregnancy at age 26, forceps were used, and a third degree laceration was sustained. Manual removal of the placenta was followed by profuse bleeding. Shortly thereafter the blood pressure was unobtainable. Following administration of 500 ml. of 5 per cent glucose in water, the blood pressure rose to 70/30. She remained hypotensive for three hours, during which time she received 1000 ml. of whole blood. After the transfusion, her blood pressure was 102/50. The hemoglobin level was 8.5 Gm. per 100 ml. on the first postpartum day. Lactation was absent. Menses did not recur. She noticed the gradual onset of intolerance to cold, dry skin, lack of perspiration, and easy fatigue. Delays in eating resulted in weakness, relieved by food. She noted progressive loss of

axillary and pubic hair and a decrease in the size of her breasts. Her libido was markedly decreased.

The patient appeared her stated age. Her weight was 56.8 Kg., height 160.3 cm., and blood pressure 88/62. The skin was smooth and extremely dry. There was marked alopecia of the lateral eyebrows; axillary hair was absent, and pubic hair was very sparse. Ophthalmoscopic findings and visual fields were normal. Pyorrhea alveolaris was noted. The thyroid gland was not palpable. The labia minora and the vaginal mucosa were markedly atrophic. The cervix and corpus uteri were small. The ovaries were not palpable. The deep tendon reflexes showed a lag of the relaxation phase.

Chest and skull roentgenograms were unremarkable. The electrocardiogram showed low voltage and nonspecific primary T-wave changes.

For the purpose of a separate investigation, prednisone only was given for about one year (thyroid therapy was withheld). At that time, the thyroidal I^{131} uptake was 11 per cent, and it increased to 24 per cent after administration of 10 units of TSH. Urinary gonadotropin titers were greater than 6.5 m.u.u. but less than 13 m.u.u. per twenty-four hours on 3 out of 4 assays.

Comment. The initial low thyroidal I^{131} uptake and the low serum PBI level indicated hypothyroidism. The low urinary excretion of 17-KS on control days was followed by an equivocal increase after administration of ACTH. Additional evidence of adrenocortical insufficiency was the marked insulin sensitivity, which was demonstrated by severe hypoglycemia during the insulin tolerance test. The absence of urinary gonadotropin in the initial assays indicated the pituitary origin of the ovarian failure.

In summary, the patient presented a classic case of postpartum pituitary necrosis with panhypopituitarism. After one year of prednisone therapy, she was clinically improved and the thyroidal I^{131} uptake and urinary excretion of gonadotropin were normal.

Case 5

G.A. (UH 239-610), a 42-year-old gravida 5, para 4, abortus 1, Negro female, was admitted in December 1955 because of weakness since her last pregnancy eleven months previously. Spontaneous delivery occurred after labor of only fifteen minutes. The extent of blood loss was undetermined. Fifteen sutures were required to close lacerations. Subsequently her breasts enlarged. Breast feeding was attempted, but bottle feeding was substituted because of deficient milk production. Later she noted weakness and weight loss. Axillary hair gradually disappeared and pubic hair did not regrow completely. Menses did not recur. Three months prior to admission she began to have episodes of nausea, vomiting, diarrhea and fever. During the month prior to admission a persistent productive cough developed; she was treated elsewhere with antibiotics for bronchopneumonia.

Physical examination revealed a seriously ill, obtunded Negro female who appeared younger than her stated age. Her weight was 43 Kg., height 157 cm., blood pressure 90/60, pulse rate 120 per minute, and oral temperature 103° F. The skin was dry, soft, smooth and extremely fine-textured. She did not appear myxedematous. The optic fundi and visual fields were normal. Pyorrhea was noted. The thyroid gland was not palpable. Axillary hair was absent, and pubic hair very sparse. There was an erosion of the cervix, but no atrophy of the vaginal mucosa or of the uterus.

Skull roentgenograms were normal. Chest roentgenograms revealed exaggerated lung markings suggestive of interstitial pneumonitis. The electrocardiogram showed minor primary T-wave changes.

The initial five weeks of hospitalization were characterized by progressive deterioration. The temperature rose to 103° F. daily. She remained weak, lethargic and bedridden. Anorexia, nausea and vomiting contributed to further weight loss. Her critical condition was not altered by administration of multiple antibiotics and supportive therapy. Postpartum pituitary necrosis was considered, but was thought unlikely in view of the normal thyroidal I^{131} uptake and the normal level of serum PBI. However, secondary adrenocortical insufficiency was established by the urinary 17-KS and 17-

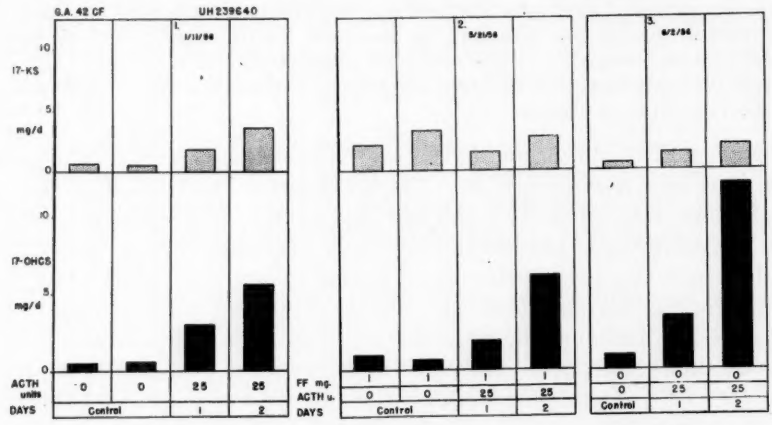

FIG. 1. *Case 5.* Urinary 17-KS and 17-OHCS determinations on 3 occasions during control days and while receiving 25 units of ACTH intravenously. During the second test the patient was receiving a daily dose of 1 mg. of 9-alpha-fluorohydrocortisone by mouth. The first test was made before, and the others after resumption of menstruation. All 3 tests demonstrate an active response to ACTH, compatible with a persistence of secondary adrenocortical insufficiency.

OHCS values (Fig. 1, first test). Cortisone acetate, 50 mg. daily, was then begun. The patient immediately became afebrile, had no further gastro-intestinal symptoms, and gained 11 pounds before discharge three weeks later.

During the first and second months after discharge, while taking 25 mg. of cortisone daily, menstrual periods recurred for the first time since parturition. This recurrence of cyclic menses prompted a second hospitalization in May 1956 for further endocrine studies. The vaginal mucosa and cervical mucus indicated an estrogenic effect. Urinary gonadotropin, previously absent, was present in an amount greater than 6.5 but less than 13 m.u.u. per twenty-four hours; and later, more than 13 but less than 52 m.u.u. Cortisone was discontinued and 9-alpha-fluorohydrocortisone (1 mg. daily) was given to prevent an adrenocortical crisis. Urinary 17-OHCS increased significantly after ACTH (Fig. 1, second test). Since 9-alpha-fluorohydrocortisone may decrease urinary 17-OHCS excretion in normal persons, presumably by pituitary inhibition of ACTH (16), all

corticoid therapy was discontinued for one week and the tests were repeated. The results indicated persistent secondary adrenocortical insufficiency (Fig. 1, test 3).

During the next four months, while not receiving cortisone, the patient had two episodes of prostration, hypotension and fever. On both occasions cortisone therapy produced prompt relief, but was discontinued. Shortly thereafter, in October 1956, she was again seen in the Outpatient Clinic; at this time the urinary excretion of 17-KS was 1.5 mg. per twenty-four hours. Cortisone acetate, 25 mg. daily, was then resumed. In October 1957 the patient was readmitted in coma. Her local physician reported she had had a sore throat with fever for several days and had discontinued cortisone. Blood pressure was unobtainable. Rectal temperature was 98.4° F. She responded quickly to intravenous fluids containing hydrocortisone hemisuccinate. For the past year and a half she has been receiving maintenance cortisone therapy; she has been asymptomatic and has menstruated regularly, usually flowing for four days each month. Endometrial biopsies on the twenty-fourth and twenty-fifth days of two recent cycles have shown proliferative endometrium with no progestational changes.

Comment. This patient's anamnesis, although recording a short labor and a traumatic delivery, did not clearly indicate excessive blood loss. Furthermore, the mammary activity in the early puerperium was not consistent with a diagnosis of postpartum pituitary necrosis. However, in women whose subsequent symptoms of hypopituitarism are only moderate, complete absence of mammary activity occurs in only about half of the cases (1, 3). Menstruation may return nine to eighteen months after delivery, and may be irregular or become almost normal (1). In this patient, cyclic menses recurred fourteen months post partum, and have persisted for three years. No evidence of ovulation was obtained, but this may have been due solely to her present age of 45 years.

In summary, thyroid functions were normal throughout the period of observation. Secondary adrenal insufficiency, shown by the urinary steroid data and the clinical course, was persistent. The return of cyclic menstruation coincided with the appearance of urinary gonadotropin. Although we can not be certain that the pituitary gland is now capable of secreting the several gonadotropins in the proper amounts and sequence for ovulation, the present clinical manifestations may be explained by an isolated deficiency of ACTH. A similar conclusion was reached by Steinberg, Shechter and Segal in a 50-year-old female with normal gonadotropin excretion (17).

Case 6

B. C. (OMRH 581), a 27-year-old Negro female, was admitted in 1956. Her third delivery at age 24 was followed by retained placenta which was manually removed twenty-four hours later. She was unconscious for many hours, and allegedly suffered excessive blood loss. She had nursed her first 2 babies, but was unable to nurse the third child due to lack of milk. Permanent amenorrhea followed. Severe occipital headaches recurred for several months. She noted easy fatigue and intolerance of cold, but had been able to perform her duties as a domestic servant.

Three weeks before admission a diagnostic dilatation and curettage was performed elsewhere. No uterine synechiae were observed and no endometrium was obtained. During the procedure the blood pressure dropped to 70/40. Following intravenous administration of vasopressors the blood pressure rose to 80/50, and it remained at this level for several days.

The patient was an asthenic, impassive Negro female who appeared her stated age. Her weight was 54.9 Kg., height 168 cm., and blood pressure 110/70. The skin was smooth and soft. The axillary hair was short and moderately sparse, but the pubic hair was normal. The optic fundi and visual fields were normal. The upper jaw was edentulous. The lower poles of the thyroid gland were palpable. The breasts were atrophic. The vaginal mucosa was markedly atrophic. The cervix, uterus, and ovaries were about one-fifth normal size.

Roentgenograms of the skull and chest were unremarkable. The electrocardiogram revealed low voltage and nonspecific primary T-wave changes.

Cortisone was started in a dosage of 25 mg. daily. Subsequently, desiccated thyroid (120 mg. daily) was added. Six months later she stated that her strength and energy had improved. Physical examination showed no significant change except for a 4-Kg. gain in weight.

Comment. The hypotension which developed during the diagnostic dilatation and curettage may have been due to adrenocortical insufficiency, and illustrates the ease with which stress may precipitate vascular collapse or coma in these patients. In cases of hypopituitarism, the failure to obtain endometrial tissue during curettage has been attributed to the extreme uterine atrophy, endometrium being represented by only a flat layer of epithelium (13).

Marked loss of axillary and pubic hair is usually considered a criterion of hypopituitarism. Nevertheless, axillary and pubic hair may be virtually normal (1, 3).

The 24-hour thyroidal I^{131} uptake was normal, yet the serum PBI level was low. A similar dissociation of these two thyroid parameters in hypopituitarism has been noted (3). Hypercholesterolemia, as noted in Case 2, is less common in secondary hypothyroidism than in the primary type.

The low excretion of 17-KS and 17-OHCS on control days followed by the marked rise during the two days of ACTH stimulation indicated secondary adrenal insufficiency. The insulin tolerance test showed insulin sensitivity and hypoglycemia unresponsiveness. Despite these additional evidences of adrenocortical insufficiency, normal water diuresis occurred in the RKP water test.

The extreme atrophy of the genital organs and the amenorrhea indicated ovarian failure. Of interest was the finding on four occasions of normal titers of urinary gonadotropin. If the ovarian failure had been due to intrinsic disease of the ovary only, a high urinary excretion of gonadotropin, as in postmenopausal patients, would be expected. The presence of urinary gonadotropin in patients with hypopituitarism has been reported (2, 3, 13,

18). Moreover it is apparent that in patients with less severe grades of hypopituitarism who subsequently experience recurrence of menstruation (1), there must be some restoration of pituitary gonadotropin secretion.

In summary, this patient exhibited incomplete hypopituitarism, presumably due to partial necrosis of the gland in the puerperium. Three years later thyroid function was nearly normal. There was evidence of secondary adrenocortical insufficiency. Ovarian failure persisted despite excretion of normal levels of urinary gonadotropin.

Case 7

T. L. (OMRH 680), a 25-year-old white female, was admitted in 1957. Following menarche at age 15 she had had regular cyclic menstruation every thirty-two days, but the scanty bleeding lasted only two to three days. The first delivery and puerperium at age 17 were unremarkable, except that she was unable to nurse the baby. Cyclic menses recurred. The second delivery at age 19 was complicated by excessive hemorrhage and a retained placenta. Five blood transfusions were given. There was no lactation, and permanent amenorrhea followed. Three months post partum she was anemic and had a low blood pressure. Subsequently, her breasts became small, and she experienced typical hot flushes. A decrease in libido was confirmed by her husband. The skin became dry. During the year before admission she had been hospitalized twice elsewhere for headache, nausea and vomiting, and had received fluids intravenously.

The patient appeared her stated age and was well-nourished. Her weight was 50.0 Kg., height 162.4 cm., and blood pressure 100/60. The skin was extremely smooth and dry. The normal capillary skin flush and tanning of exposed surfaces were lacking. Ophthalmoscopic findings and visual fields were normal. Several teeth were carious and abscessed. The thyroid gland was not palpable. The nipples and areolae were lightly pigmented. Axillary and pubic hair was extremely sparse. Pelvic examination revealed atrophy of the clitoris and labia, which resembled those of a prepubertal female. The vaginal mucosa was thin and pale and showed only occasional rugae. The corpus uteri was tiny. The ovaries were not palpable.

Skull and chest roentgenograms were normal, but those of the lumbosacral spine showed moderate osteoporosis. An electrocardiogram was normal.

Cortisone acetate, 25 mg. daily, was given in a therapeutic trial. During eight months her weight increased by 9.6 Kg. She felt better and stronger than at any time since her last pregnancy. She observed that tanning of the skin now occurred on exposure to the sun. Menses did not recur during cortisone therapy. Withdrawal bleeding did not follow a five-day trial of oral progestational medication, nor did it follow two courses of intensive oral estrogen therapy.

Comment. Euthyroidism was shown by the normal thyroidal I^{131} uptake and the normal level of serum PBI. Urinary 17-KS excretion was low on control days and increased slightly during ACTH stimulation. Additional evidence of adrenocortical insufficiency was the absence of diuresis after a water load. Insulin sensitivity was observed during the insulin tolerance test, but the response to induced hypoglycemia was normal.

The history of scanty menses prior to the pregnancies and the inability to nurse the first baby suggested some previous endocrine abnormality.

Hot flushes are usually interpreted as due to primary ovarian failure. However, hot flushes have been reported in postpartum pituitary necrosis (1, 3) and following surgical hypophysectomy in menstruating or castrated females (19). In this patient, titers of urinary gonadotropin were repeatedly higher than normal, but not as high as usually found in postmenopausal females. The absence of withdrawal bleeding following estrogen therapy suggests that severe endometrial atrophy occurred post partum and persisted. It is a matter of speculation whether the uterine atrophy was due to pelvic inflammation or to a severe but temporary deficiency of pituitary gonadotropins.[1]

In summary, the classic history and the laboratory data suggest postpartum pituitary necrosis, manifested at the time of study by moderate adrenocortical hypofunction due to adrenocorticotropic deficiency.

DISCUSSION

As in previous series of patients with hypopituitarism (1, 20), asthenia and easy fatigue were prominent symptoms. The frequent occurrence of dental caries and pyorrhea has also been mentioned; all of the present patients were edentulous or had dental caries and pyorrhea. The electrocardiograms in the patients with a low level of serum PBI exhibited primary T-wave changes and low voltage. The electrocardiogram was normal in Case 5 and showed only minor primary T-wave changes in Case 7, the 2 cases in which both the PBI and the thyroidal I^{131} uptake were normal.

Thyroid function, as evaluated by the thyroidal uptake of I^{131} and the serum level of PBI, varied from normal to markedly deficient. Combinations of findings with these two tests in individual cases were: 1) low I^{131} uptake unresponsive to TSH, and a low level of PBI; 2) low I^{131} uptake increasing to normal after TSH, and a low level PBI; 3) normal I^{131} uptake, and a low level of PBI; and 4) normal I^{131} uptake, and a normal level of PBI. The first combination, discussed in the Comment on Case 1, is presumably due to irreversible thyroidal atrophy and fibrosis following severe TSH deficiency of long duration. The second combination, which includes increased I^{121} uptake after exogenous TSH stimulation, is the expected response in secondary hypothyroidism. The third combination of normal I^{131} uptake and low serum PBI concentration has been observed occasionally in postpartum pituitary necrosis (3), and in hypopituitarism due to tumors (21). Werner *et al.* also noted low-normal I^{131} uptakes in hypopituitarism (22). Jeffries *et al.* showed that a normal thyroidal I^{131} uptake does not imply the ability to supply normal amounts of thyroid hormone; in hypopituitarism a decreased formation or storage of hormone may occur

[1] Since acceptance of this manuscript, uterine bleeding occurred following administration of 10 mg. of conjugated equine estrogens daily for ten weeks.

in the presence of intact iodide-trapping mechanisms (21). The fourth combination of a normal level of serum PBI and a normal thyroidal I^{131} uptake might be attributed to essentially normal pituitary production of TSH. However, even in these cases of hypopituitarism thyroid reserve may be impaired, as shown by the failure of exogenous TSH to increase the serum level of PBI (21).

Decreased thyroid function in hypopituitarism may partially protect against acute adrenocortical insufficiency. Clinicians have long recognized that replacement of glucocorticoids should occur before replacement of thyroid hormones. Furthermore, the plasma half-time of hydrocortisone may be increased in myxedema and decreased in thyrotoxicosis (23). Hypothyroidism was present in our first 4 patients, and no episodes suggesting acute adrenocortical insufficiency had occurred before admission, although urinary 17-KS excretion was very low. In contrast, in the fifth patient, there was normal thyroid function but severe adrenocortical insufficiency. In the last 2 patients, thyroid function was nearly normal and episodes suggesting acute adrenal insufficiency had occurred.

Urinary 17-KS excretion was low in all patients on control days; the response during two days of ACTH stimulation varied from no change to a definite increase. Urinary 17-OHCS (determined in 3 patients) showed a more rapid and greater response to ACTH.

With 1 exception (Case 5), each patient had severe atrophy of the genital organs and permanent amenorrhea since the last delivery. Within the group, urinary gonadotropin excretion varied from nil to moderately elevated. In individual patients, gonadotropin excretion varied as follows: 1) persistently absent, 2) absent to normal, 3) normal, and 4) normal to moderately elevated. Although normal or slightly elevated excretion of gonadotropin was detected initially in Cases 3, 6 and 7, and later in Case 4, ovarian insufficiency was present as shown by severe atrophy of the genital organs and amenorrhea. Physiologic pituitary secretion of gonadotropins was therefore unlikely.

A correlation was observed between the urinary gonadotropin assays and the thyroidal I^{131} uptakes. Two of the 4 patients with clinical hypothyroidism and low I^{131} uptakes did not excrete detectable amounts of gonadotropin at any time. Two assays in Case 3 were negative and 2 others were low-normal. In Case 4, urinary gonadotropin was initially absent, and the thyroidal I^{131} uptake was very low; however, one year later when this woman was clinically improved during prednisone therapy, the I^{131} uptake was normal and urinary gonadotropin was detected on several occasions, although amenorrhea persisted. Two of the 3 patients with normal thyroidal I^{131} uptakes had normal or slightly elevated excretion of gonadotropin persistently, whereas the third patient did not excrete de-

tectable amounts of gonadotropin initially, but excreted normal amounts after the recurrence of menstruation.

The question of whether or not the target glands fail in a consistent sequence in association with progressive destruction of the adenohypophysis must be considered. In 34 patients with anterior pituitary deficiency from various causes, Peters *et al.* concluded that the sequence of organs undergoing secondary functional impairment was the gonads, the thyroid gland and then the adrenal cortex (24). Adrenocortical functions were evaluated by the ability to conserve salt and to maintain the proper concentration of blood sugar. However, it is likely that these patients had low adrenocortical reserve, as it was noted that a satisfactory response to these tests was no assurance that collapse would not follow a serious injury, illness or operation. Since then the demonstration of the importance of aldosterone in maintaining normal levels of serum electrolytes and of its partial independence from pituitary control would cast doubt upon the validity of using salt conservation as a sensitive and crucial means of evaluating adrenocortical function in hypopituitarism (25).

The effects of graded hypophysectomy on thyroid, gonadal and adrenocortical functions have been studied in the dog by Ganong and Hume (26). Surgical removal of 75 per cent of the anterior pituitary gland did not result in detectable endocrine abnormality. Removal of 97 to 99 per cent was associated with atrophy of all 3 target glands. Removal of 75 to 95 per cent of the anterior lobe was associated with gonadal insufficiency alone, or gonadal and thyroidal insufficiency together. Isolated hypofunction of the thyroid or the adrenal cortex was not observed; nevertheless, the authors did not feel that reports of selective failure of the pituitary to secrete thyrotropin or ACTH in human subjects were necessarily in disagreement with their animal data.

Cases of apparent selective failure of the pituitary to secrete one or several tropic hormones have been reported. Maddock *et al.* observed 4 males who were considered to have deficient ACTH and gonadotropin secretion, but normal thyrotropin secretion (27). Selective TSH deficiency has been reported in an elderly diabetic female (28) and in a young man (29).

Our cases may be divided into 2 broad syndromes. The first 4 patients were characterized by hypothyroidism, adrenocortical hypofunction, lack of urinary gonadotropin, and amenorrhea. The last 3 patients exhibited nearly normal thyroid function, adrenocortical hypofunction, and normal to slightly elevated excretion of gonadotropin, with or without amenorrhea. One patient (Case 5) showed failure of the adrenal cortex alone.

In the absence of assays of TSH and ACTH, the commonly accepted basis for inferring a deficiency of a specific tropic hormone is to demonstrate hypofunction of a target gland and then to show a response to its

tropic hormone. By the use of such reasoning it may be assumed that in some cases of postpartum pituitary necrosis there is decreased secretion of both ACTH and TSH, and in others a decreased secretion of ACTH with nearly normal secretion of TSH. Gonadotropin was apparently low or absent in all the patients with decreased secretion of both ACTH and TSH. On the other hand, gonadotropin was present or even increased in the patients with normal secretion of TSH but decreased secretion of ACTH.

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THE DEVELOPMENT OF HORMONAL BIOSYNTHESIS IN HUMAN FETAL THYROIDS

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ABSTRACT

Analysis of the iodinated components of the thyroids of fetuses from mothers given radioiodine prior to legal abortion (Japan) indicated ability to concentrate iodide at 14 weeks and ability to synthesize organic iodine compounds at 15 to 19 weeks. In 2 anencephalic fetuses the thyroid was able to synthesize the normal thyroidal iodine compounds.

THE human fetal thyroid has been reported (1–3) to be capable of concentrating radioiodine between the third and fourth months of pregnancy. Studies of fetal thyroids of animals (4–7) have indicated that the ability to concentrate iodide is acquired first, then the ability to form monoiodotyrosine, next diiodotyrosine and finally thyroxine. This chronology is similar to that currently accepted for the biosynthesis of thyroidal organic iodine compounds (8).

The present study, undertaken to determine the development of thyroxine synthesis in the human fetus, indicates that at 14 weeks iodide can be accumulated and that only later (by 19 weeks) can thyroxine be synthesized.

METHODS

Seven pregnant women who wanted legal abortions (Japan) and 2 pregnant women with anencephalic fetuses were given 50–150 μc. of radioactive iodine from twenty-four to forty-eight hours before delivery. Intact fetuses were obtained and carefully measured for the estimation of age in weeks. The fetal thyroid was then carefully removed, homogenized, and subjected to hydrolysis with 4 per cent pancreatin and 4 per cent trypsin for twenty-four hours. The hydrolysate was concentrated to dryness and redissolved in 2*N* NH_4 OH (1 ml.). An aliquot (20–100 μl.) was then chromatographed. Chromatograms were developed in a butanol-acetic acid solvent system (B A) by ascending chromatography. Strips of Toyo filter paper No. 50, 2 cm. to 4 cm. wide and 35 cm. long, were used in chromatography. Carrier monoiodotyrosine, diiodotyrosine, thyroxine and triiodothyronine were applied to the paper. After chromatography, the strips were radioautographed and stained; each centimeter was counted in a scintillation crystal detector.

RESULTS

Thyroids from 7 fetuses ranging in age from 14 to 28 weeks (Table 1), and 2 anencephalic fetuses aged 40 weeks, were studied. In 2 other fetuses,

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aged 5 weeks, the tissue containing the thyroid did not exhibit a detectable amount of radioactivity. In the normal fetus aged 14 weeks, the thyroid concentrated only inorganic iodide, indicating that no detectable binding of iodine had taken place. At nineteen weeks, however, thyroxine was being synthesized in easily detectable amounts (15 per cent of the total radioiodine). Unfortunately the ratio of monoiodotyrosine to diiodotyrosine (MIT/DIT) could not be determined because of poor chromatographic separation. In the 4 normal fetuses aged 22, 24, 26 and 28 weeks, the

TABLE 1. CHROMATOGRAPHIC ANALYSIS OF RADIOIODINE COMPOUNDS IN THE FETAL THYROID

Fetus				Distribution of radioactivity (% of total I^{131})*									
				Ascending system (BA)						Descending system (AN)			
No.	Sex	Length (mm.)	Age (wks.)	O	I^-	MIT	DIT	MIT/DIT	T_4+T_3	O	I^-	T_3	T_4
1	M	92	14		†								
2	M	142	19	10.2	10.1	42‡			15.8	50.1	10.2	16.1	0.5
3	F	174	22	10.0	15.1	22.1	44.5	0.49	5.0	80.1	10.2	5.0	0.7
4	F	161	23	10.1	10.2	30.5	5.5	5.5†	30.5	50.2	15.2	20.1	3.0
5	M	182	24	8.8	7.2	16.5	36.2	0.46	22.1	55.7	11.0	19.3	4.0
6	M	201	26	3.9	8.1	25.8	55.5	0.47	5.0	71.8	15.0	4.4	1.0
7	M	222	28	4.1	10.1	26.9	52.2	0.52	7.0	73.2	15.0	6.5	1.2

* Total activity does not equal 100% because only the discrete spots were counted, not the interspot activity. Total uptakes ranged from 0–2% of the dose given to the mother.

† An abnormal fetus (see text).

‡ Poor separation of MIT and DIT chromatographically.

MIT/DIT ratios were between 0.46 and 0.52. In the abnormal fetus (No. 4) with a huge abdomen containing ascitic fluid, rudimentary bowels, and a huge liver and spleen (fetal hydrops), the MIT/DIT ratio was 5.5. Thyroxine in the normal fetuses was 4.4–19.3 per cent, and triiodothyronine 0.5–4.0 per cent of the total radioactivity (Table 1). In abnormal fetus No. 4 the concentration of thyroxine was 30.5 per cent. This high concentration of thyroxine with a high ratio of monoiodotyrosine to diiodotyrosine has not been explained. It is possible that some unknown substance traveled with the same Rf as thyroxine, or that, in the presence of a slow conversion of monoiodotyrosine to diiodotyrosine, an alternate pathway for thyroxine synthesis was developed. The chromatograms for the two 40-week-old anencephalic fetuses showed radioiodine at the origin, and as iodide and thyroxine. These 2 thyroids were analyzed only by descending chromatography. Histologic examination in one of these anencephalic fetuses failed to reveal any pituitary tissue.

DISCUSSION

The thyroid of the chick embryo is able to concentrate iodide at $7\frac{1}{2}$ days, and to synthesize monoiodotyrosine at $8\frac{1}{2}$ days, diiodotyrosine at $9\frac{1}{4}$ days

and thyroxine at 9¾ days (7). The results presented here may indicate a similar sequential development of the ability to trap iodide (some time before 14 weeks) and to organify iodine and make thyroxine (some time between 14 and 19 weeks) in the human fetal thyroid. If the development of the ability to make monoiodotyrosine precedes the ability to make diiodotyrosine, as in the chick, then the ratio of monoiodotyrosine to diiodotyrosine must initially be high. Unfortunately, in our study, an earlier evaluation was made impossible by poor chromatography, but it was observed that by 22 weeks the ratio of monoiodotyrosine to diiodotyrosine was normal in the human fetal thyroid. The single case of a high ratio of monoiodotyrosine to diiodotyrosine occurred in an abnormal fetus and does not necessarily indicate the normal sequence for the synthesis of iodinated tyrosines. This study does not offer any evidence on whether separate enzymes are required for the synthesis of diiodotyrosine and monoiodotyrosine.

It is of particular interest that in the absence of pituitary tissue (anencephaly) the fetal thyroid develops the ability to concentrate iodide and make thyroxine. Presumably the biosynthetic mechanism of the thyroid can be developed in the absence of thyrotropic hormone from the pituitary (9).

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ELEVATED THYROIDAL I^{131} UPTAKE IN THE ABSENCE OF GOITER IN ISOLATED VENEZUELAN INDIANS

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ABSTRACT

The 24-hour thyroidal uptake of I^{131} was measured in 53 well nourished Indians from the Alto Ventuari region, Amazonas territory, Venezuela. These Indians had had little or no contact with white men and, with 1 exception, their thyroids were either nonpalpable or normal in size. The average I^{131} uptake was 70.8 per cent of the administered dose; 45 of the cases had uptakes of more than 50 per cent of the dose. Uptakes of 6 members of the expedition were within normal limits. If it be assumed that the demonstrated thyroidal avidity for iodine means iodine deficiency, the findings would suggest that such deficiency may exist without giving rise to goiter.

IN PREVIOUS studies (1–4) of thyroidal radioiodine (I^{131}) uptake in endemic goiter regions, a number of subjects without goiter have been shown to have a high uptake, of the order of 80 to 95 per cent of the administered dose. It appeared to us of interest to investigate I^{131} uptake in an isolated region where iodine deficiency could be expected, but where no goiter had been previously reported.

MATERIAL AND METHODS

The site chosen for investigation was the savanna of Kąkuri, near the sources of the Ventuari river, in the Amazon territory of Venezuela.

As far as we are able to ascertain, only one scientific expedition had been previously in the region,[1] that of Koch-Grünberg in 1911 (5). The savanna of Kakuri (Fig. 1) lies in the midst of virgin tropical forests, near the Ventuari river, and is normally uninhabited, except by occasional nomad Indians. A preliminary air exploration was carried out by Dr. Pierre Couret and an Indian interpreter of the Maquiritare tribe, Isaías Rodríguez. The 2 observers were able to land at Kakuri and with the help of local Indians

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[1] However, a rudimentary airfield had been established by the Venezuelan Charting Service (Cartografía Nacional).

proceeded to clean up the area so that larger planes could land and a base camp be established.

The rest of the expedition arrived a few weeks later in DC-3 planes, which permitted the transportation of heavy laboratory equipment. By traveling up and down the river in Indian embarkations, it was possible to

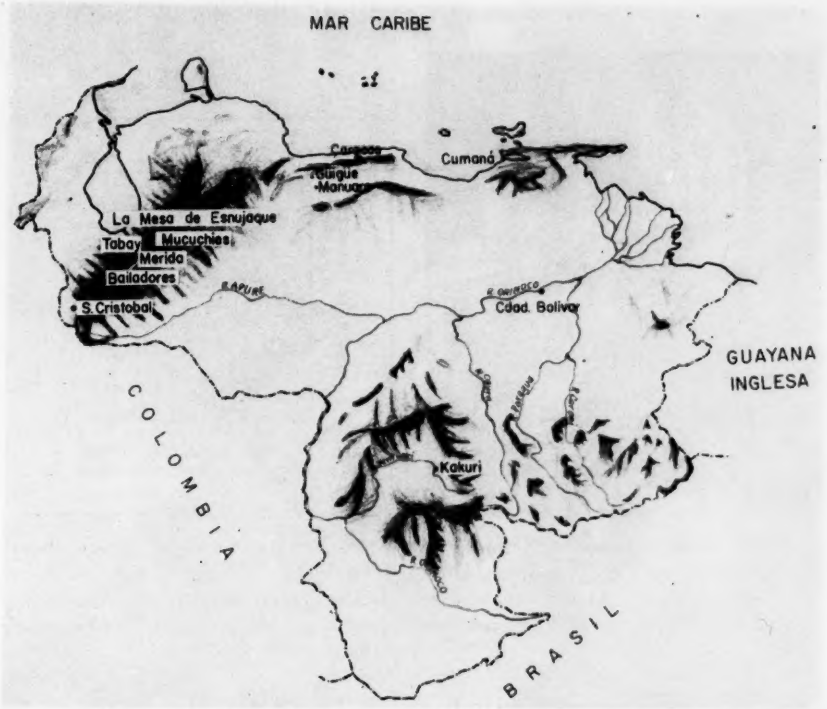

FIG. 1. Map of Venezuela. The chief endemic goiter region lies to the west in the Andes, around Mérida and Bailadores. The present study was carried out in Kakuri, Amazon territory, a wild isolated wooded area close to the river Ventuari.

gather at the camp some 110 Indians. These Indians belonged to two different tribes: 1) the Maquiritares, possibly issued from old Caribe stock, who are strong, well-nourished river Indians, masters of rudimentary weaving crafts and good builders of barks, and 2) the Guaharibos, a very savage, nomad forest tribe, smaller and thinner than the Maquiritare, yet well nourished. The food of these Indians consisted of dried manioc (yucca), fish and whatever wild animal could occasionally be hunted. As far as could be ascertained, mixture with white men is minimal among the

Maquiritares and non-existent among the Guaharibos. A complete physical examination was made of the 89 Indians studied. Cardiologic, epidemiologic, anthropologic and zoologic data, as well as geographic data were collected, and will be reported elsewhere by the respective groups.

Radioiodine[2] (I^{131}) was administered orally in a dose of 25 to 50 μc. Thyroidal radioactivity was counted in 53 of the Indians as described previously (1), twenty-four hours after administration (Fig. 2). The thyroidal

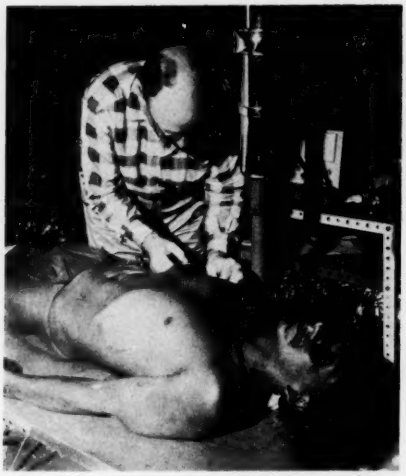

FIG. 2. Determination of thyroidal I^{131} uptake on Subject #38, Maquiritare tribe. The thyroid was not palpable; the uptake was 69 per cent of the administered dose of I^{131}.

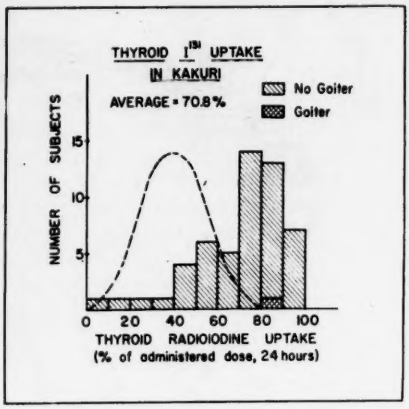

FIG. 3. Histogram of 24-hour thyroid I^{131} uptake in Kakuri. The dotted line indicates the distribution of uptake in Boston, Mass., and in Caracas, the Venezuelan capital.

I^{131} uptake was also measured in Kakuri in 7 menbers of the expedition. The thyroid was not palpable, or barely so, in 72 of the Indians. It was palpable but apparently normal in 14, and palpable and slightly enlarged in 2. In 1 girl, approximately 12 years of age, the thyroid was visibly enlarged and there was a round, firm nodule in the left lobe.

RESULTS

The results are shown in Figure 3. The dotted line indicates the distribution of thyroidal I^{131} uptake in Boston, Massachusetts (6) and in Caracas (7). It may be seen that the uptake among the Indians was markedly deviated toward the upper levels, although a few uptakes were normal and

[2] Abbott Laboratories, Oak Ridge.

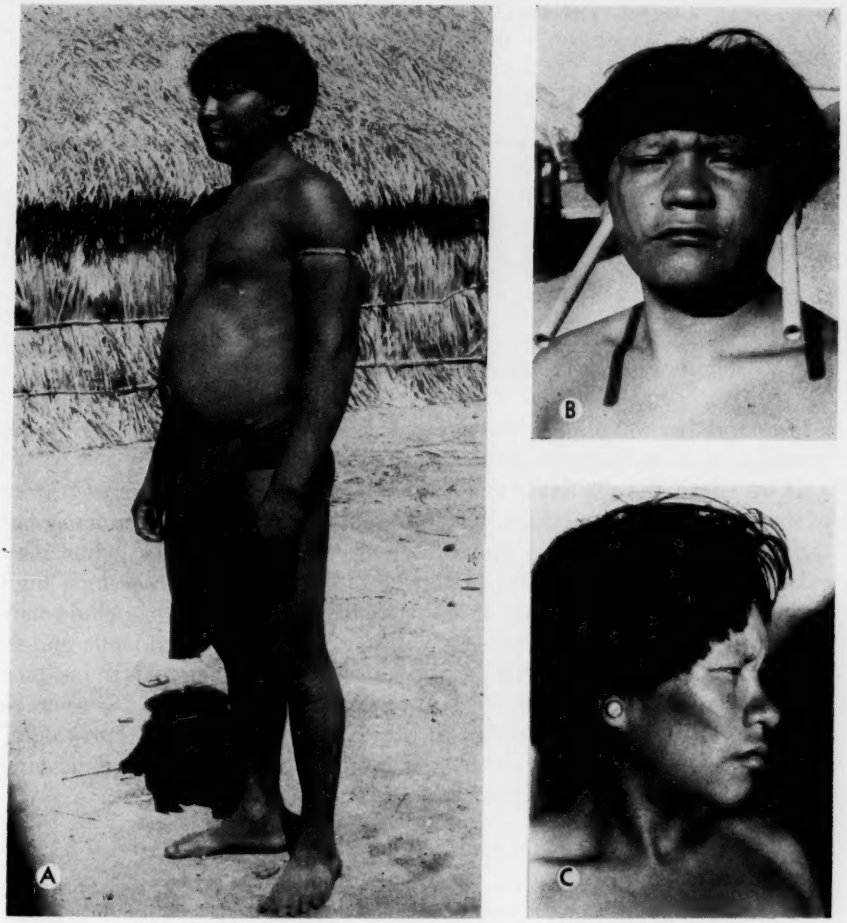

FIG. 4. **A**) Subject #76, Maquiritare tribe; notice good nutritional state; thyroid barely palpable; I^{131} uptake 98 per cent. **B**) same subject. **C**) Subject #59, Guaharibo tribe; thyroid palpable but not enlarged; I^{131} uptake 97 per cent of dose.

even low. The results of uptake determinations on 7 members of the expedition are shown in Table 1.

DISCUSSION

The average uptake of 70.8 per cent was similar to that of 74 per cent obtained in Bailadores, a region of high endemicity (1). There was no apparent relationship between the clinical size of the thyroid and I^{131} avidity. In

TABLE 1. THYROIDAL I^{131} UPTAKE IN ADULT MALE MEMBERS OF THE EXPEDITION

Subject	I^{131} uptake (% of dose)
J.M.C.	25
A.R.	31
A.B.	38
A.D.	31
A.G.	37
B.Z.	36
I.R.*	37

* Maquiritare Indian guide who has been living in Caracas for fifteen years.

a large number of subjects the thyroid was not palpable, but the uptake was high, as in Subjects 38, 59 and 76 (Figs. 2 and 4).

If we admit the relatively small sample studied as being representative of the larger, widely scattered, sparse Indian population of the same region, the present results suggest that a high thyroidal avidity for iodine may exist in a general population, without goiter. Again, if we assume high thyroidal avidity to be a sign of iodine deficiency, we might conclude that such lack of iodine can be present without giving rise to endemic goiter. Iodine deficiency in these Indians may not be profound enough to produce goiter; this is unlikely, however, since in other regions where the average iodine uptake is distinctly smaller than in Kakuri (3, 4), goiter is prevalent. On the other hand, it may be that, in the human being, iodine deficiency is a necessary but not a sufficient cause of endemic goiter.

Acknowledgments

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DEFECT OF THE IODINATING SYSTEM IN CONGENITAL GOITROUS CRETINISM: REPORT OF A CASE WITH BIOCHEMICAL STUDIES*

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ABSTRACT

A study of a 15½-year-old male with goitrous cretinism is reported. The patient's thyroid gland concentrated I^{131} well but released the accumulated I^{131} following oral administration of thiocyanate. Thyroid tissue obtained by thyroidectomy was homogenized and fractionated by differential centrifugation into 900×g and 40,000×g sediments. Each fraction was suspended in Krebs-Ringer bicarbonate buffer, pH 8.4, and incubated with I^{131} and iodide or I^{131}, iodide and an H_2O_2-generating system. No monoiodotyrosine (MIT) was found on chromatograms of the enzymatically hydrolyzed incubation mixtures. Normal human thyroid tissue under the same conditions formed MIT, and the addition of an H_2O_2-generating system effected augmentation of MIT formation. Three possibilities are considered for the abnormal mechanism in the patient's thyroid gland: 1) an iodinase defect, 2) defective formation of "active" iodide, or 3) an abnormal receptor protein (thyroglobulin). The first possibility is favored in this case.

THE congenital goitrous cretin is an hypothyroid newborn infant in whom the functional capacity of the thyroid gland for forming biologically active hormone is impaired (1, 2). Although such a gland may trap I^{131} quite actively (1–4), the conversion of the trapped iodide to thyroid hormone is defective (1–4). Recent studies have shown that the thyroid gland of a goitrous cretin may exhibit one of at least three defects:

1. In one group of cases the thyroid gland was capable of iodinating tyrosine, but was unable to form iodinated thyronines. It was thought that in these cretins there was decreased activity of the "coupling enzyme," an enzyme believed to be involved in the coupling of iodinated tyrosines to form thyroid hormones (5).

2. In another group of patients, the thyroid also iodinated tyrosines,

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forming monoiodotyrosine (MIT) and diiodotyrosine (DIT), but there was a deficiency in the amount of thyroid hormone formed. MIT and DIT were readily detected in the serum and urine as well as in hydrolyzates of the thyroid gland, after the administration of I^{131}. There was a marked decrease of deiodinase activity in the thyroid gland (6, 7). This enzyme might be thought of as an efficiency device in the normal gland, for by deiodinating free MIT and DIT, it conserves both the iodide and the tyrosine and in effect directs traffic toward hormone production (8).

It is of interest that congenital goitrous cretins, and to a lesser degree other hypothyroid patients show decreased activity of an extrathyroidal (peripheral) tyrosine deiodinating enzyme (7, 9). The activity of this enzyme, however, was found in most instances to rise toward normal after substitution therapy with thyroid substance (7, 9, 10).

3. In another group, the thyroid gland avidly trapped I^{131} but discharged it more or less completely when thiocyanate was given (1, 2). These patients showed a marked inability to iodinate tyrosine. This deficiency was considered to be due to a defect in an "oxidase system" (5).

In the present paper a case of congenital goitrous cretinism is reported in which the thyroid gland avidly trapped iodide but discharged it upon administration of thiocyanate. In order to study this defect of iodide conversion, cell-free preparations of the patient's thyroid gland were used. Cell-free preparations of human and sheep thyroid glands have been found to convert iodide into an organic form and produce MIT (11, 12). Such preparations of normal human and sheep thyroid tissue exhibit enhanced iodinating activity when incubated with an exogenous oxidative system (13, 14).

CASE REPORT

J. H. T. (HLH A 30521) was 15½ years old when he underwent thyroidectomy. He was referred to the Pediatric Endocrine Clinic of the Johns Hopkins Hospital because of marked cretinism since infancy. Treatment with desiccated (USP) thyroid was instituted at the age of 14 months, but administration was irregular after the age of 6½ years. Treatment was discontinued six months prior to the operation; soon thereafter the child's neck enlarged progressively.

The patient had 1 sibling, who was euthyroid and healthy. There was no history of thyroid disorders, hereditary diseases, or consanguinity in the family.

Physical examination showed a quiet, pale, retarded white male. The facies were infantile, with marked cretinoid, myxedematous features (Fig. 1). He was retarded mentally, and had an intelligence quotient of less than 60. He was physically underdeveloped, with a height of 50 inches and a height-age of 8 years. On palpation, the thyroid gland was firm, nodular and enlarged. He was 18 pounds overweight for his height; this was due mostly to myxedema, since he lost 10 pounds within a month after thyroid therapy was instituted. The pulse was 60 per minute and the blood pressure was 90/60 mm. Hg.

X-ray examination showed marked retardation of osseous development, a bone age

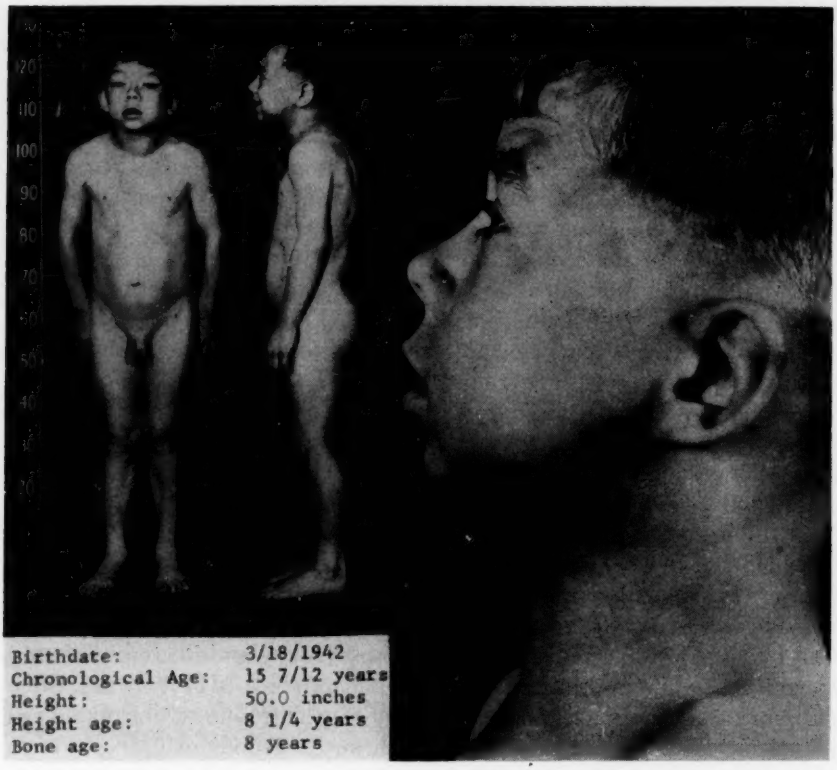

FIG. 1. On the left, the front and side views of the patient are shown against the grid graded in centimeters. The characteristic habitus is demonstrated. On the right, the profile of the head and neck shows the goiter and the infantile, cretinoid features of the face.

of 8½ years, and dysgenesis of many epiphyses. Radiologic and fluoroscopic examination of the chest showed moderate enlargement of the cardiac silhouette; the cardiac pulsations were reduced. Electrocardiographic examination showed marked reduction in voltage.

The level of blood hemoglobin was 11.5 Gm., serum cholesterol 580 mg., serum protein-bound iodine (PBI)[1] 1.3 μg., and serum total iodine[2] 11 μg. per 100 ml.

Clinical investigations. There was a rapid uptake of I^{131} by the thyroid gland each time following an oral dose and an intravenous dose of I^{131}, given three months apart (Fig. 2). Although the peak concentration of I^{131} occurred in less than six hours, the radio-

[1] Determined by the Bio-Science Laboratories, Los Angeles, California.

[2] We were unable to explain why the level of serum total iodine was so high, unless it was caused by contamination of the serum sample. Only 1 determination was performed.

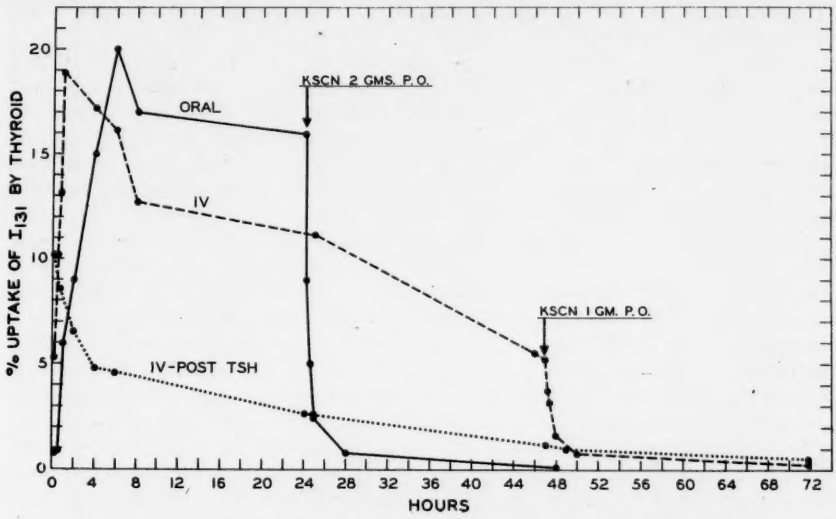

FIG. 2. Showing the rapid thyroidal uptake of both orally and intravenously (IV) administered I^{131} with prompt release of radioactivity upon administration of thiocyanate (KSCN), and the decreased uptake with prompt and spontaneous release of radioactivity following administration of TSH. Thiocyanate had been administered three weeks prior to the TSH test.

activity in the neck was sustained for twenty-four to forty-eight hours, but disappeared promptly after respective oral doses of 1 Gm. and 2 Gm. of potassium thiocyanate. In order to evaluate the effect of thyrotropic hormone (TSH) on iodide uptake, TSH was given intramuscularly (1 USP unit daily for three successive days) prior to the administration of an intravenous dose of I^{131}. There was a decrease in the thyroidal uptake of I^{131}, followed by a spontaneous discharge of the radioactivity from the gland. This apparent inhibition of I^{131} uptake and spontaneous discharge may have been spurious, resulting from a residual portion of the thiocyanate given three weeks previously.

Almost all the radioactivity administered was recovered in the urine as iodide-I^{131} within seventy-two hours, as detected by paper chromatography.[3] There was no conversion of plasma I^{131} into PBI^{131} (15). Samples of plasma collected at 4, 8, 12, 24, 36 and 48 hours before and after administration of thiocyanate were extracted with acidified n-butanol (10) and chromatographed. All the radioactivity which was recovered on the paper chromatogram was found in the iodide region. No iodinated tyrosines or thyronines were detected.

The activity of peripheral deiodinase was significantly impaired. A dose of 50 μc. of *l*-DIT labeled with I^{131} was administered intravenously. In a four-hour urine specimen collected after the injection, 38.2 μc. of the radioactivity was recovered, of which 22.5 per cent was DIT-I^{131}, 2.5 per cent MIT-I^{131}, and 75 per cent iodide-I^{131}. Thus 8.6 μc. or

[3] Solvents used for paper chromatography were n-butanol:2*N* NH_4OH (1:1); and n-butanol:2*N* acetic acid (1:1).

17.2 per cent of the administered dose of DIT-I^{131} was recovered unchanged in the four-hour sample of urine (6, 16).

Thyroidectomy. On December 29, 1957, thyroidectomy was performed. The thyroid gland weighed 15 Gm. and was pale brown, firm and nodular. Histologically, it showed marked proliferation of the acinar columnar epithelium with very little colloid (Fig. 3). In some sections, this proliferation of acinar epithelium was so profound that it resembled carcinomatous change (Fig. 3), though no carcinoma was present. There were scattered areas of fibrosis, but the gross nodular pattern of the gland was not distinctly demonstrated in the microscopic sections.

Study (in vitro) *of thyroid tissue.* The thyroid tissue was collected at the operating room, placed in an iced container and kept chilled through all steps of preparation. It was weighed, minced, and then homogenized with Krebs-Ringer bicarbonate buffer solution, pH 8.4, in a Waring Blendor. All procedures were carried out in the cold room at a temperature of 4°C. The homogenate was strained through a fine mesh, and then partitioned by differential centrifugation in an International refrigerated centrifuge into 2 fractions, I and II, corresponding to the "nuclear" fraction and the mitochondrial and microsomal fraction respectively. Each of the fractions was resuspended separately in Krebs-Ringer bicarbonate buffer solution (pH 8.4) so that the equivalent of 0.4 Gm. of the original tissue in 1 ml. of the buffer solution was placed in each glass-stoppered flask. Carrier-free I^{131} (5 μc. in 10 μl. of solution)[4] and 10 μl. of potassium iodide solution containing 2 μg. of iodide were added. A mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled briefly into the solution and the flask was immediately stoppered. It was placed in a constant temperature water-bath at 37° C. for two hours, with frequent agitation, Incubation was terminated by chilling the flask in an ice-water bath. Propylthiouracil (10 μl. of 0.1 *M* solution) was added to prevent further incorporation of I^{131}.

In order to test the effect of an H_2O_2-generating system on the iodinating activity of the thyroidal fractions, either glucose-oxidase or xanthine-oxidase with the respective substrate was added to the incubation flask containing the thyroid fraction suspended in buffer, with iodide and I^{131}, just before the beginning of incubation. A 10-μl. volume of a 0.02 *M* solution of glucose was used as substrate for 10 μl. of glucose oxidase[5] solution containing 1.5 units; and a 10-μl. volume of 0.05 *M* solution of hypoxanthine was used as substrate for 1 unit of xanthine-oxidase[6] in 10 μl.

Aliquots of 25 μl. of the incubation mixture were placed on Whatman #1 filter paper, 2 inch-wide strips, for chromatography. The papers were run overnight in a descending direction in jars maintained at a constant temperature of 37° C. Representative samples were run in parallel in 3 solvents: n-butanol:ethanol:2*N* NH_4OH (100:20:40), n-butanol:2*N* NH_4OH (1:1), and n-butanol:2*N* acetic acid (1:1); the former was found the most satisfactory. The chromatograms were dried and radioautographed. The areas showing radioactivity were cut out and counted in a well-type scintillation counter. In order to avoid errors due to trailing of radioactivity on the chromatogram, those areas showing no radioactivity by the radioautograph were cut into strips 1 cm. wide and were counted in the same way.

Similar aliquots of the incubation mixture were chromatographed after: 1) acidification with 1*N* HCL, 2) addition of 10 μl. of $10^{-3}M$ thiocyanate solution, and 3) enzy-

[4] The I^{131} used was the intravenous preparation of E. R. Squibb & Sons, New Brunswick, N. J.

[5] From Takamine Laboratories, Clifton, N. J.

[6] Worthington Biochemical Corp., Freehold, N. J.

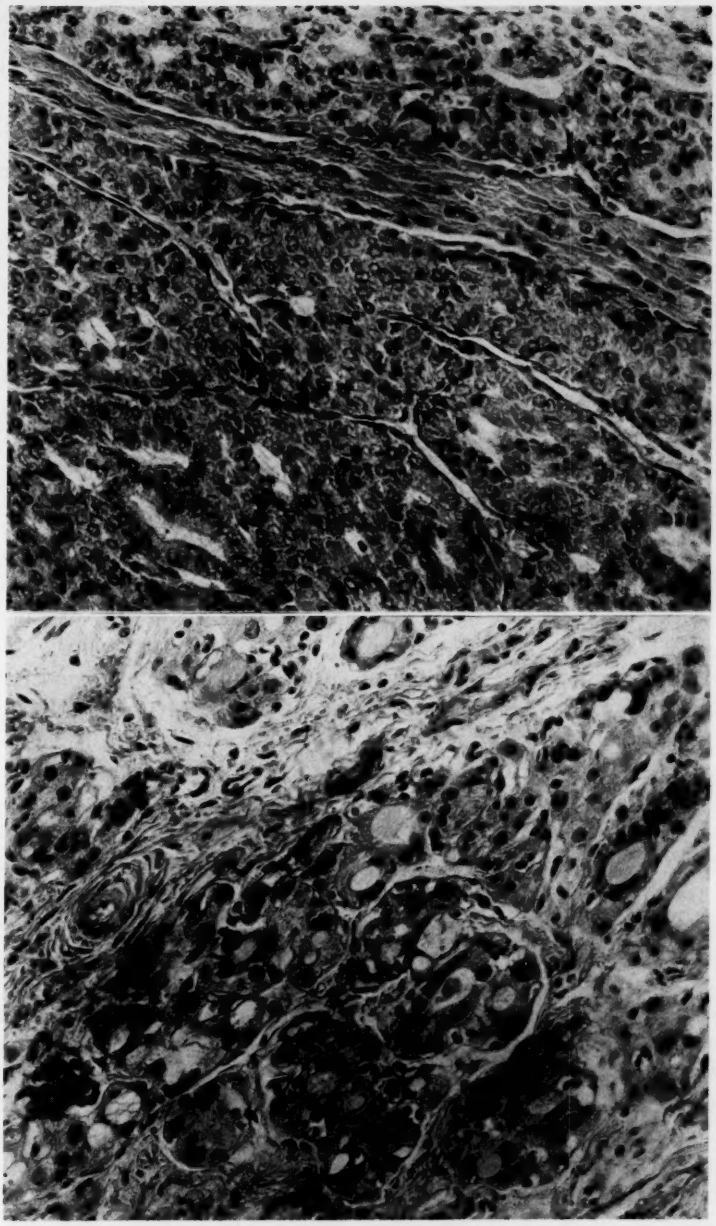

FIG. 3. Photomicrographs of sections of different areas of the thyroid gland which was removed. The upper section demonstrates marked proliferation of the acinar columnar epithelium. The lower section demonstrates extreme proliferation of the columnar epithelium, to an extent resembling carcinoma.

matic hydrolysis by incubation with crude pancreatic extract (Pangestin) for four hours at 37° C.

Control studies were made on biopsy specimens of thyroid tissue from 5 euthyroid subjects who were undergoing neck dissection, removal of a solitary "cold" nodule from the thyroid, or exploration for a parathyroid adenoma. The thyroid tissue from each subject was processed individually, according to the described procedure.

RESULTS

Normal controls. On incubating I^{131} with either fraction I or II from normal human thyroid gland, 3 areas of radioactivity were consistently shown on the radioautographs following chromatographing of an aliquot of the incubation mixture on paper using an alkaline solvent system (butanol:ammonia or butanol:ethanol:ammonia). The radioactivity remaining at the origin of the chromatograph was designated as the "O" component. The second and largest area on the radioautograph was the unchanged iodide, designated as the "I" component, and the third and fastest moving component was designated "U" (U_2 of Taurog *et al.* (11)). The average proportion of the total radioactivity on the radioautograph in the "O" and the "U" components was 4.5 per cent and 6.3 per cent respectively when fraction I was used, and 2.2 per cent and 1.8 per cent respectively when fraction II was used (Fig. 4, column A).

Acidification of the incubation mixture resulted in a 20–30 per cent decrease in the radioactivity of the "O" component, whereas the "U" component completely disappeared. The loss was accounted for by an increase in the iodide spot. Thiocyanate effected the disappearance of the "U" component only, but the product, though having an Rf similar to iodide, was distinct from it. Thiocyanate had no effect on the radioactivity at the origin. When the incubation mixture was digested with Pangestin, about 80 per cent of the radioactivity which was at the origin, "O," was recovered as MIT (monoiodotyrosine) when chromatographed. Enzymatic digestion also resulted in the almost complete disappearance of the "U" component which was recovered in the iodide area (Fig. 4, column 2).

The major part of the "O" component was apparently an organic iodide compound. The "U" component, on the other hand, was an unstable iodide compound, the nature of which was not determined. It had an Rf value similar to that of thyroxine but could be distinguished by its acid lability, reaction to thiocyanate, and disappearance during the enzymatic digestion procedure. It should be noted, too, that the "U" component was not found on chromatographs run in acidic solvents (butanol-acetic acid).

Normal controls+H_2O_2-generating system. When either fraction I or fraction II from normal human thyroid tissue was incubated with I^{131}, iodide and an H_2O_2-generating system (glucose+glucose-oxidase or hypoxanthine+xanthine-oxidase), there was an increase of about 70–80 per

cent in the relative yield of component "O," with a corresponding increase in the amount of MIT formed when the incubation mixture was enzymatically hydrolyzed (Fig. 4, column C).

Patient's thyroid. On chromatograms from the incubation mixture of either fraction I or fraction II of the patient's thyroid, both components "O" and "U" were present in proportions of 4.5 and 6.3 per cent respectively in incubation mixtures of fraction I (Fig. 5, column A), and 0.6 and 0.9 per cent respectively in mixtures of fraction II. Both components "O" and "U," however, were unstable at low pH or when hydrolyzed with Pangestin; their radioactivity was quantitatively and qualitatively added to that of iodide. No MIT was formed to be detected on the chromato-

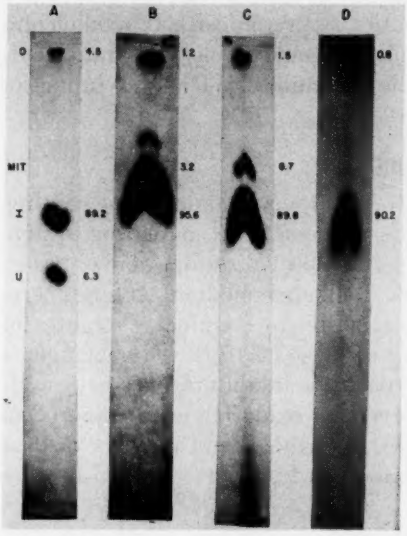

FIG. 4. A, B, and C are radioaudiographs of fraction I from normal human thyroid incubated with I^{131} and chromatographed with the butanol:ethanol:NH_4OH system* and run for seventeen hours. A is the undigested control. B is the control digested with Pangestin. C (glucose and glucose oxidase added) was digested with Pangestin. D is fraction I of the patient's thyroid gland incubated with I^{131}, glucose and glucose oxidase, and digested with Pangestin. D shows no MIT formation.

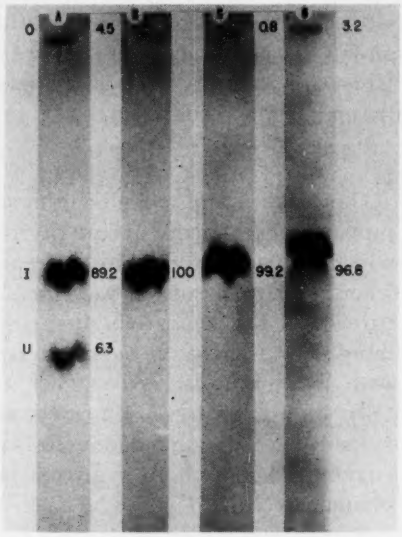

FIG. 5. Radioautographs of chromatograms of patient's thyroid fraction I (A) following incubation with I^{131} and iodide, (B) when subjected to low pH, (C) when hydrolyzed with Pangestin, and (D) following addition of 10 μl. of 1/1000 thiocyanate solution. All chromatograms were run in butanol:ethanol:NH_4OH solvent for twenty-four hours.

* Except B, which was run in the butanol:NH_4OH system.

graphs (Fig. 5, column B and C). When 10 μl. of 1/1000 thiocyanate solution was added to the incubation mixture, the "O" component was unchanged, whereas the "U" component behaved as in the case of normal thyroid controls (Fig. 5, column D).

Patient's thyroid fractions+H_2O_2-generating system. The incubation of either fraction I or fraction II of the patient's thyroid with I^{131}, iodide and an H_2O_2-generating system did not result in an acid stable "O" component. Upon enzymatic hydrolysis, there was no detectable MIT on the chromatograms (Fig 4, column D).

DISCUSSION

Our studies and those of others (11, 13, 14) have shown that cell-free preparations of mammalian thyroid tissue possess an iodinating system which converts iodide into an organic form. Optimal results were obtained when these preparations were incubated in alkaline medium, at a temperature of 37°C., in an oxygen-enriched atmosphere for ninety minutes or more (11). Iodination was inhibited by catalase (11, 14), reducing agents such as thiouria derivatives (13), and oxidative inhibitors such as cyanide (13). Iodination was augmented, however, by oxidative agents and coenzymes such as flavin mononucleotide (13), by glucose and xanthine oxidase enzymes (12), and by hydrogen peroxide slowly generated from a cellophane bag (14).

When either glucose-oxidase or xanthine-oxidase was incubated with I^{131} alone or with boiled cell-free fractions of human thyroid, no conversion of I^{131} into the organic form occurred during the incubation period used here, and MIT was not produced (12). This suggests that the exogenous oxidase, which generates minute quantities of H_2O_2, can augment iodination only under the conditions used, when the thyroid enzymes are functional. Keston (17) and others (18–20), however, were able to iodinate milk protein or casein by incubating it with iodide, xanthine-oxidase and hypoxanthine. Roche *et al.* (16) also could form MIT and DIT from tyrosine incubated with H_2O_2 and iodide in acid medium. In all of these experiments iodination was dependent upon the formation of iodine via iodide oxidation, which was readily detectable in solution. It was shown, too, that elemental iodine alone, without oxidative agents, could iodinate tyrosine (16) and albumin (21). Iodination as effected by thyroid cell-free fractions, on the other hand, was inhibited by a high concentration of H_2O_2 such as is used in the systems of Keston (17) and others (13, 14). Also, it was shown by Serif and Kirkwood (14) that thyroid cell-free fractions failed to utilize elemental iodine for enzymatic iodination.

Since slowly generated H_2O_2 enhanced iodination by thyroid cell-free fractions, but did not initiate it independently (12), it would appear that

in the process of iodination by such fractions, an oxidative step is necessary only to convert the iodide into an "active" form acceptable by such fractions for iodination. In the incubation mixture of thyroid cell-free fractions, it has been impossible to determine the exact nature of this "active" form of iodide. It was not of the nature of elemental iodine, because we could not recover radioactivity by carbon tetrachloride extraction from such incubation mixtures. No evidence has been adduced to implicate the "U" component recovered on the chromatograms in the conversion of iodide or its further incorporation. It is likely that once iodide is activated, a specific system (iodinase) in the thyroid cell-free fractions catalyzes incorporation into the organic form. This would agree with the hypothesis of Kirkwood and co-workers (22, 23) that two steps are involved in the process of iodination *in vitro* by cell-free fractions of thyroid tissue: 1) an oxidative step which activates iodide into an active iodide ion, probably a hypoiodous acidium ion (IOH_2^+) or an iodinium ion (I^+), rather than elemental iodine (I_2), and 2) an iodinating step which attaches the "active" iodide into the tyrosyl residues, as demonstrated in the following schematic formula:

$$\underset{\text{iodide}}{I^-} \xrightarrow{\text{oxidase}} \underset{\text{"active"}}{(I)} \xrightarrow{\text{iodinase}} \underset{\text{"organic"}}{(Ty\text{-}I)}.$$

A study of the gland of the patient reported here was undertaken in the hope of being able to demonstrate the site of the defect by the demonstration of iodination in the thyroid homogenate when a system which slowly generated H_2O_2 was added, but not in its absence. This result would have implicated a step or a series of reactions required for activation of iodide. Although we could obtain stimulation of iodination in normal human thyroid homogenates when an H_2O_2-generating system was added, *i.e.*, glucase+glucose-oxidase or hypoxanthine+xanthine-oxidase, no iodination could be shown in the homogenate of this patient's gland under identical conditions. One of several interpretations is possible: 1) this patient has a deficiency in iodinase activity, 2) a catalytic amount of an "activated iodide" must be formed before an H_2O_2-generating system can augment iodination occurring via the normal pathway, or 3) the acceptor protein was abnormal. The last possibility has a prototype in studies demonstrating an abnormal protein (non-thyroglobulin) in homogenates of human thyroid cancer (24) and transplantable rat thyroid tumors (25). The defect in the case under study would of necessity be of a different nature, inasmuch as in the studies cited the abnormal protein was found to be iodinated; moreover, in the goitrous patient with such an abnormal protein, there was considerable thyroidal retention of I^{131} which was unaffected by

administration of thiocyanate (26). The evidence at hand favors the first possibility, but no conclusive evidence contrary to the other possibilities has been obtained.

The occurrence of a heritable defect involving the iodide-activating system, or the iodinase system, or the synthesis of an abnormal receptor protein (thyroglobulin) would seem equally likely from the genetic standpoint. Indeed, it would be surprising if all 3 types are not found eventually. With the methods of clinical investigation currently available, it would be impossible to distinguish between these defects. Such differentiation would require refinements of the *in vitro* procedures and of other methods for studying the physical and chemical properties of thyroglobulin.

Acknowledgment

We should like to express our appreciation to Dr. Lawson Wilkins for his advice and encouragement.

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ADRENOCORTICAL RESPONSE TO SURGICAL OPERATIONS IN PATIENTS TREATED WITH CORTICOSTEROIDS OR CORTICOTROPIN PRIOR TO SURGERY

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ABSTRACT

The free plasma 17-OHCS response to preoperative intravenous ACTH and to surgical operation was measured in 6 patients who had been receiving prolonged corticosteroid therapy; in 4, the plasma 17-OHCS conjugate levels were also determined. The plasma 17-OHCS response to preoperative ACTH was diminished in all 6 patients, but in 2 of them the free plasma 17-OHCS levels noted postoperatively were within the normal range expected following surgery. In contrast to the foregoing group were 2 patients who had received long-acting ACTH preparations for ulcerative colitis. Their plasma 17-OHCS response to both ACTH and to total colectomy was normal. It is suggested that patients who have been treated only with ACTH prior to surgery may be expected to have a normal pituitary-adrenal response to operation.

THE administration of adrenocortical steroids in a great variety of disease states is now so widespread that it has become essential for the clinician to have a detailed understanding of the physiologic and chemical changes brought about by these potent agents. One of the most important effects of these steroids—suppression of endogenous pituitary-adrenal function—has been recognized since Ingle's demonstration that long-term administration of adrenocortical extract results in atrophy of the adrenal cortex in animals (1). Numerous reports have appeared emphasizing the occurrence of sudden vascular collapse and sometimes death following surgery, not only in patients who were receiving corticosteroid therapy at the time of operation but also in patients who had discontinued steroid treatment weeks or months before (2–5). These observations suggested the importance of comparing the effects of maximal-dose infusions of ACTH with those of surgical operations on the plasma levels of free and of conjugated 17-hydroxycorticosteroids in patients who have been receiving either corticosteroid or corticotropin (ACTH) therapy prior to surgery. In

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this report, data dealing with these aspects of the effect of surgery on the adrenal cortex will be presented.

METHODS AND MATERIALS

Ten patients ranging in age from 25 to 64 years were selected so that the series would include major and minor elective surgical procedures. Only 1 subject in this study was female. The majority of operations were performed under cyclopropane or ether anesthesia. Spinal anesthesia was employed in 2 cases.

Nine patients were receiving either corticosteroid or corticotropin therapy immediately prior to surgery. One subject had discontinued prednisone five months before operation. The adrenocortical response to a six-hour saline infusion of 30 I.U. of ACTH was measured one to seven days preoperatively in 9 patients. Hormone therapy was discontinued for twenty-four hours before the performance of the ACTH test. An identical ACTH infusion was administered one or two days postoperatively in 5 of these patients. In a tenth subject the plasma 17-hydroxycorticosteroid (17-OHCS) response to ACTH-gel was determined on the day preceding operation. Supplementary adrenal steroid therapy was given immediately before operation to cover the actual surgical procedure in 7 patients. Table 1 shows the hormones and dosages employed.

Samples of venous blood were collected before, and at two, four and six hours after the beginning of all ACTH infusions. Armour ACTH was used in these studies. On the day of surgery, blood was drawn prior to anesthesia, at the end of the operation, and at two and/or four hours postoperatively in 8 patients; blood was also obtained during the surgical procedure in 5 of them.

Plasma free 17-hydroxycorticosteroids were measured by a modification of the method of Nelson and Samuels (6). Plasma conjugated 17-hydroxycorticosteroids were determined by the method of Bongiovanni (7).

RESULTS

A summary of pertinent clinical data on the patients selected for study may be seen in Table 1. In this table are included the dosages of the various hormones received by these patients, both as maintenance therapy for their medical disease and as supplementary therapy in preparation for surgery.

I. *Preoperative Plasma 17-OHCS Response to Infusion of ACTH*

The effect of a standard infusion of ACTH on the level of free plasma 17-OHCS was studied in 9 patients preoperatively (Table 2). In 5 of them there was an opportunity to repeat the studies on either the first or second postoperative day.

It is apparent that the preoperative free plasma 17-OHCS response to ACTH in Patients 1–8 (the corticosteroid-treated group) was diminished in comparison with the mean values for the normal control group. The preoperative conjugated plasma 17-OHCS response to ACTH was also found to be decreased in those patients on whom these measurements were performed (Table 3).

TABLE 1. MAINTENANCE HORMONE THERAPY FOR MEDICAL DISEASE AND SUPPLEMENTARY HORMONE THERAPY IN PREPARATION FOR SURGICAL OPERATION

Subjects*	Disease	Maintenance hormone therapy					Supplementary hormone therapy			
					Last dose		Preoperative		Postoperative	
		Hormone	Daily dosage (range)	Duration	Amount	Time preop.	Hormone	Dosage (mg.)	Hormone	Dosage† (mg.)
1) J.K., 64	Bronchial asthma	Triamcinolone; Cortisone	2– 24 mg. 50–100 mg.	7 mos. 12 days	2 mg. 50 mg.	48 hrs. 48 hrs.	Fluorohydrocortisone Fluorohydrocortisone	6 6	Cortisone Cortisone	100 100
2) F.W., 48	Neurodermatitis	Cortisone; 6-Methylprednisolone	100–300 mg. 24 mg.	7 yrs. 3 mos.	200 mg. 4.0 mg.	3 mos. 48 hrs.	None		Prednisolone	50
3) E.V., 33	Rheumatoid arthritis	Triamcinolone	6– 8 mg.	6 mos.	2 mg.	16 hrs.	None		Prednisolone	50
4) C.L., 42	Myelo-radiculitis	Prednisone	10– 15 mg.	6 mos.	10 mg.	5 mos.	Cortisone	500	Cortisone	100
5) J.G., 42	Bronchial asthma	Cortisone; Prednisolone	100–200 mg. 20 mg.	4 yrs. 12 mos.	150 mg. 5 mg.	12 mos. 48 hrs.	Fluorohydrocortisone	6	Cortisone	100
6) R.L., 63	Bronchial asthma	Dexamethasone; Prednisone	3– 5 mg. 20 mg.	5 mos. 5 wks.	3 mg. 5 mg.	2 wks. 48 hrs.	Fluorohydrocortisone	6	Cortisone	100
7) M.C., 55	Rheumatoid arthritis	Prednisolone	7.5– 15 mg.	18 mos.	2.5 mg.	48 hrs.	Cortisone	200	Cortisone	100
8) W.M., 25	Pericarditis	Prednisone	20– 40 mg.	4 mos.	5.0 mg.	96 hrs.	Cortisone	300	Cortisone	200
9) W.D., 32	Ulcerative colitis	Zinc-ACTH	70– 80 I.U.	3 mos.	35 I.U.	24 hrs.	None		None	
10) F.C., 35	Ulcerative colitis	ACTH-Gel	60–120 I.U.	3 wks.	20 I.U.	24 hrs.	Fluorohydrocortisone	6	None	

* The data after each case number are the patient's initials and age.
† Administered parenterally on day of operation.

TABLE 2. EFFECT OF ACTH INFUSIONS OF FREE PLASMA 17-OHCS LEVELS

Subjects	Infusion, days preop.	Preoperative 17-OHCS (μg./100 ml.)				Infusion, days postop.	Postoperative 17-OHCS (μg./100 ml.)			
		Control	Hours				Control	Hours		
			2	4	6			2	4	6
1. J.K.	1	8	11	14	21					
2. F.W.	1	7	6	8	10					
3. E.V.	2	13	16	18	17					
4. C.L.	4	19	25	29	34	1	36	47	55	
5. J.G.	1	14	20	27	28	2	25	38	46	43
6. R.L.	1	15	16	21	25					
7. M.C.	2	12	11	20	18	1	31	48	53	53
8. W.M.	4	9	16	20	23	2	26	35	48	47
9. W.D.	7	27	42	47	45	1	40	53	69	68
10. F.C.*	1	45		73						
Normal (15 subjects)	Mean	18.4	33.0	40.2	46.1					
	S.E.M.	±2.1	±3.0	±3.3	±3.6					

* This patient received 20 I.U. of ACTH-gel intramuscularly. All other patients received a six-hour infusion of 30 I.U. of ACTH.

TABLE 3. EFFECT OF ACTH INFUSIONS ON CONJUGATED PLASMA 17-OHCS LEVELS

Subjects	Preoperative 17-OHCS (μg./100 ml.)			Postoperative 17-OHCS (μg./100 ml.)		
	Control	Hours		Control	Hours	
		4	6		4	6
1. J.K.	11	13	16			
2. F.W.	17	13	14			
3. E.V.	11	10	12			
5. J.G.	7	9	12	14	19	21
7. M.C.	9	12	13	21	27	30
8. W.M.	14	17	19	23	28	32
9. W.D.	21	27	29	32	38	41
10. F.C.*	29	42				
Normal (15 subjects)	Mean 14.9	24.8	30.7			
	S.E.M. ±1.8	±2.4	±2.9			

* This patient received 20 I.U. of ACTH-gel intramuscularly. All other patients received a six-hour infusion of 30 I.U. of ACTH.

In contrast to the foregoing subjects, the 2 patients (Nos. 9 and 10) who had received long-acting corticotropin preparations prior to surgery demonstrated a normal and a slightly exaggerated plasma 17-OHCS response to intravenous ACTH and to ACTH-gel,[1] respectively.

II. *Plasma 17-OHCS Response to Surgery*

Patients who had received corticosteroids prior to surgery

The free and conjugated plasma 17-OHCS levels in response to surgical operations in all of the patients studied are recorded in Table 4. During the immediate postoperative period, the free plasma 17-OHCS levels reached

TABLE 4. EFFECT OF SURGICAL OPERATIONS ON FREE AND CONJUGATED PLASMA 17-OHCS LEVELS

Subjects	Operation	Free plasma 17-OHCS (μg./100 ml.)					Conjugated plasma 17-OHCS (μg./100 ml.)				
		Before anesthesia	During operation	Hours postop.			Before anesthesia	During operation	Hours postop.		
				0	2	4			0	2	4
1. J.K.	Subtotal colectomy	6	21	25	22		10		11	14	
	Closure of colostomy	12		12	15		28		25	31	
2. F.W.	Tenorrhaphy	4		4	7		12		13	16	
3. E.V.	Dilation and curettage	11		10	11		9		7	10	
4. C.L.	Transurethral resection	29		32	38						
5. J.G.	Cholecystectomy	11	26	33		27	5	4	10		9
6. R.L.	Transurethral resection	11	9	19	28	25					
9. W.D.	Total colectomy	25	64	73	65		20	22	33	35	
10. F.C.	Total colectomy	28	61	66		105	14	19	25		46

in Patients 1, 2, 3 and 6 were definitely less than we have observed in patients with normal adrenal function who underwent similar surgical procedures (8, 9). In Patients 4 and 5, levels of free plasma 17-OHCS developed following surgery which were within our normal range for these operations (8, 9). However, it is difficult to interpret the elevated free plasma 17-OHCS values observed in Patient 4, for he had received 100 mg. of cortisone acetate intramuscularly on the evening before operation. The conjugated plasma 17-OHCS response to surgery also appeared to be diminished in Patients 1, 2, 3 and 5. In evaluating these results, it is important to bear in mind that in patients with normal adrenal function elevated levels of conjugated plasma 17-OHCS may not develop postoperatively. Some patients have impaired ability to conjugate circulating free 17-OHCS during the immediate postoperative period (9, 10).

[1] In our experience, the free and the conjugated plasma 17-OHCS levels four hours following the intramuscular injection of 20 I.U. of ACTH-gel to normal adults have not exceeded 45 μg. and 30 μg. per 100 ml., respectively.

Patients who had received corticotropin prior to surgery

It is of interest that in Patients 9 and 10, who had received only corticotropin prior to operation, high levels of both free and conjugated plasma 17-OHCS developed following total colectomy performed for ulcerative colitis. Patient 9 did not receive any supplementary hormone therapy to cover the surgical procedure and had a completely uneventful postoperative course.

III. *Comparison of Effects of ACTH Infusion on Plasma 17-OHCS Levels, Preoperatively and at Varying Intervals Postoperatively*

In 3 patients who had received corticosteroid therapy prior to surgery, higher levels of both free and conjugated plasma 17-OHCS developed in response to ACTH administered either one or two days postoperatively than in response to identical infusions given preoperatively (Tables 2 and 3). A similar observation was made in Patient W.D., who had been treated with zinc-ACTH for three months prior to study.

In interpreting these results, it is evident that the plasma 17-OHCS levels immediately preceding the ACTH infusion were higher postoperatively than preoperatively. However, the increment in free 17-OHCS concentration in response to ACTH was greater postoperatively than preoperatively in every instance. This same phenomenon was also seen with respect to conjugated plasma 17-OHCS, although to a lesser degree than for free 17-OHCS.

The following 2 cases are presented in detail because in both subjects postoperative complications developed which frequently have been reported to occur in patients who have received cortisosteroid therapy prior to surgery (2–5):

Case #1

Clinical summary. E. V., a 33-year-old female, entered the hospital with metrorrhagia of several weeks' duration. She had been taking triamcinolone daily for rheumatoid arthritis for six months prior to admission (Table 1). A six-hour ACTH infusion was administered two days preoperatively. The patient received her usual oral daily dose of triamcinolone (6 mg.) on the day preceding surgery. A diagnostic dilation and curettage was performed under cyclopropane anesthesia, without incident. Following operation, 2 liters of 5 per cent glucose-in-water was infused over a five-hour period. At the completion of this infusion, the patient complained of generalized muscular weakness, became nauseated, and vomited several times. A 20-mm. drop in systolic blood pressure was recorded. The patient was given 50 mg. of prednisolone hemisuccinate in 500 ml. of physiologic sodium chloride intravenously over a two-hour preiod; there was a corresponding rapid improvement in her clinical condition (Fig. 1).

Endocrine summary. The infusion of ACTH two days preoperatively produced a rise in the level of free plasma 17-OHCS from 13 μg. to 18 μg. per 100 ml., and in the level of conjugated plasma 17-OHCS from 11 μg. to 12 μg. per 100 ml. No elevation in plasma

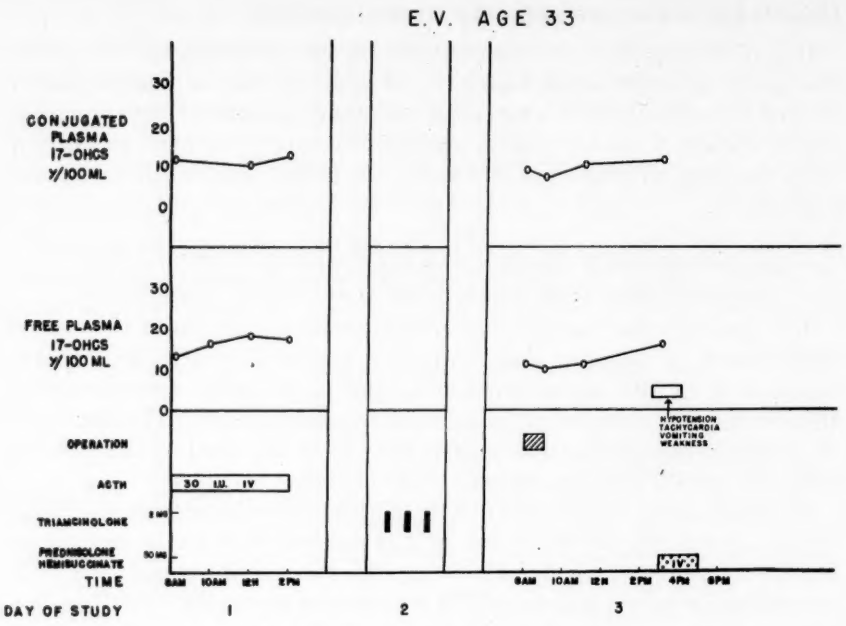

FIG. 1. Response of plasma 17-OHCS levels to ACTH, corticosteroids and surgery in Subject E.V. The hours and days of study are plotted along the abscissa. The dosages of hormones and the concentrations of free and conjugated plasma 17-hydroxycorticosteroids are indicated along the lower and the upper part of the ordinate, respectively.

17-OHCS concentration occurred in response to operation and cyclopropane anesthesia (Table 4). At the time of the mild adrenal crisis, the free plasma 17-OHCS level was 14 μg. per 100 ml. (Fig. 1).

Comment. Six months of triamcinolone therapy resulted in profound adrenocortical suppression in this subject, as indicated by the minimal plasma 17-OHCS response both to ACTH and to surgery. The appearance of a mild adrenocortical crisis postoperatively may have been related not only to the operative procedure but also to the infusion of 2 liters of 5 per cent glucose-in-water during the immediate postoperative period, for adrenocortical insufficiency renders patients extremely susceptible to water intoxication (4).

Case #2

Clinical summary. C. L., a 42-year-old male, entered the hospital with urinary obstructions of several months' duration due to a contracted vesical neck. He had been suffering from myeloradiculitis of unknown etiology for one year and had received a six-month course of prednisone therapy for this condition. Prednisone was discontinued

five months prior to admission (Table 1). The patient received a six-hour infusion of ACTH four days preoperatively and was prepared for surgery with intramuscular cortisone acetate, being given 500 mg. over the three-day period immediately prior to operation (Table 1). A transurethral resection of the contracted vesical neck was performed under ether anesthesia without incident. A shaking chill developed two hours postoperatively, followed by a fever of 39.5° C. His blood pressure remained stable throughout this entire episode. However, the patient was given 100 mg. of cortisone acetate as routine postoperative steroid coverage.

Mild urethral bleeding was observed on the first postoperative day while the patient was receiving an infusion of ACTH. One hour later, there was sudden onset of shock (Fig. 2). The ACTH was discontinued and 1 unit of blood was rapidly transfused. The patient remained in a state of shock as urethral bleeding continued. An infusion of hydrocortisone hemisuccinate was started and 100 mg. of cortisone acetate was ad-administered intramuscularly. The patient was taken to the operating room, where fulguration of several vesical bleeding areas was performed. During surgery he received 1 additional unit of blood and 100 mg. of hydrocortisone hemisuccinate. Coincident with these therapeutic procedures, the blood pressure and pulse returned to normal levels. Daily injections of cortisone acetate were given for one week postoperatively.

Endocrine summary. The infusion of ACTH four days preoperatively produced an elevation in the level of free plasma 17-OHCS from 19 μg. to 34 μg. per 100 ml. The administration of cortisone acetate preoperatively resulted in a free plasma 17-OHCS concentration of 29 μg. per 100 ml. immediately prior to anesthesia. Two hours postoperatively the free plasma 17-OHCS level was 38 μg. per 100 ml. (Table 4). On the first postoperative day, free plasma 17-OHCS concentration rose from 36 μg. to 55 μg. per 100 ml. in response to a four-hour infusion of ACTH. The latter plasma steroid value was obtained at the moment shock occurred.

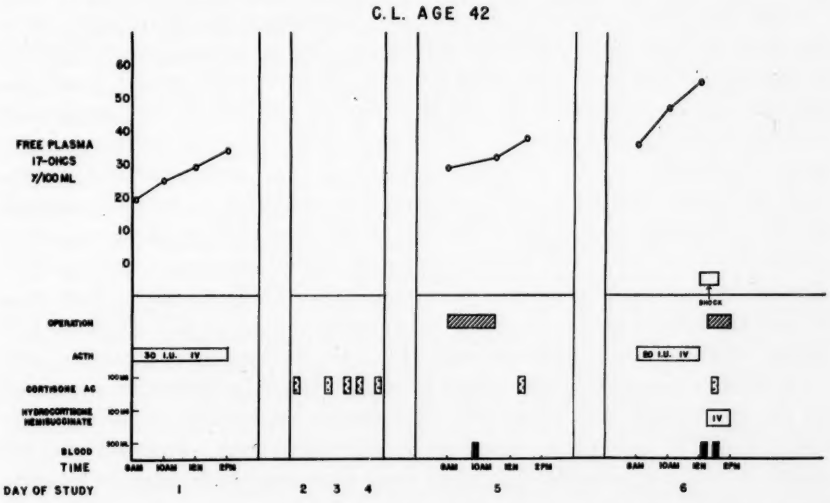

FIG. 2. Response of plasma 17-OHCS levels to ACTH, corticosteroids and surgery in Subject C.L. The data are plotted as indicated in the legend to Figure 1.

Comment. This subject's plasma 17-OHCS response to ACTH preoperatively was just below our normal range of adrenocortical responsiveness to ACTH. This finding is of clinical interest because the patient had not received any corticosteroid therapy for five months prior to this study. His adrenocortical response to surgery is difficult to interpret, because large doses of cortisone acetate were administered preoperatively. The observation that his blood pressure remained stable in spite of a severe shaking chill and fever two hours postoperatively suggests that a free plasma 17-OHCS level of 38 μg. per 100 ml. was adequate to maintain normal vascular responsiveness to stress.

The occurrence of shock on the first postoperative day, while the papatient was receiving ACTH, requires explanation. At the time, adrenocortical insufficiency was considered to be present because of the lack of blood pressure response to 1 unit of blood; accordingly, hydrocortisone was given intravenously. The finding of a free plasma 17-OHCS level of 55 μg. per 100 ml. at the moment shock appeared is evidence against this thesis. A more likely possibility is that this patient's central nervous system disease rendered his vasomotor reflexes relatively insensitive to loss of blood. Fulguration of the bleeding vessels combined with an additional unit of blood was probably more effective therapeutically than administration of hydrocortisone in combating the peripheral vascular collapse.

DISCUSSION

From the data presented in this study, it appears that prolonged corticosteroid therapy in man reduces the adrenocortical capacity to respond to exogenous ACTH and to surgical trauma. These results furnish biochemical support for numerous clinical observations concerning the dangers of surgical stress in patients who have been receiving corticosteroid therapy prior to operation (2–5). In both animals and man, prolonged administration of adrenal corticosteroids has been demonstrated to produce adrenal atrophy by suppressing the secretion of corticotropin from the anterior pituitary (1, 11, 12). The recent synthetic steroid analogues are not only capable of causing the inhibition of pituitary ACTH secretion, but on a weight-for-weight basis, they are much more potent in this respect than either cortisone or hydrocortisone (13, 14). Various investigators have been unable to correlate the degree of adrenocortical suppression produced by corticosteroids with either the total dosage or the duration of therapy (15, 16). This individual variation in adrenocortical responsiveness explains why some patients who have been receiving corticosteroid therapy react badly to surgery, whereas others do not.

The duration of adrenal suppression after cessation of steroid therapy is unknown. Certain patients may respond normally to exogenous ACTH

within one or two days of stopping treatment, whereas others require weeks of ACTH administration to restore normal adrenocortical responsiveness (13, 16). Even after corticosteroid therapy has been discontinued for as long as four to twenty-four months, irreversible shock may develop after minor surgical procedures (3, 17). In this regard, we considered the sudden appearance of shock in Patient C.L. as a manifestation of adrenocortical insufficiency. However, the finding of a free plasma 17-OHCS level of 55 μg. per 100 ml. at the time shock occurred makes this explanation untenable. The response of persistent hypotension during or following surgery to intravenous hydrocortisone is not necessarily an objective criterion of adrenocortical insufficiency, for the use of hydrocortisone in supraphysiologic amounts during shock may potentiate the effect of vasopressor agents in patients with normal adrenocortical function (18, 19).

Although it is well accepted that corticosteroid-induced adrenal atrophy renders the patient vulnerable to surgical stress, the role of ACTH-induced pituitary suppression in this regard is not clearly established. Case reports suggest that patients who have received corticotropin therapy prior to surgery may be as susceptible to vascular collapse during operation as those who have received corticosteroids (17, 20). Unfortunately, no measurements of adrenocortical hormones were presented with these clinical observations.

Our results furnish indirect evidence that the anterior pituitary is capable of secreting ACTH in response to surgical trauma in patients treated with either corticosteroids or corticotropin prior to operation. First, in several of our patients there was evidence of an adrenocortical response to surgery as measured by a rise in the levels of both free and conjugated plasma 17-OHCS. Amatruda and coworkers have demonstrated a normal rise in plasma 17-OHCS concentration during the stress of hypoglycemia in patients who had received prolonged ACTH and steroid therapy, thus suggesting that hypothalamic-pituitary-adrenal function was normal in these patients (21). Holub *et al.* have found that the administration of large doses of cortisone acetate to rats for one week reduces the pituitary ACTH concentration by 61 per cent (22). However, the application of a scalding stress to these animals resulted in a 50 per cent reduction in concentration of the remaining ACTH, indicating the ability of a pituitary with a reduced ACTH content to secrete ACTH in response to a stressful stimulus.

Second, the infusion of ACTH on the first or second postoperative day in 4 of our patients produced higher free and conjugated plasma 17-OHCS levels than did an identical infusion preoperatively. Although the pre-ACTH plasma 17-OHCS levels were elevated postoperatively, the increment in 17-OHCS concentration in response to ACTH was greater postoperatively than preoperatively in every instance. It is conceivable that

impaired metabolism of hydrocortisone postoperatively could result in higher free plasma 17-OHCS levels. However, this phenomenon is usually present only during the immediate postoperative period and has not been observed on the first or second postoperative day (9). In patients with normal adrenal function, a standard infusion of ACTH results in higher plasma 17-OHCS levels postoperatively than preoperatively (23). We have interpreted this observation as evidence that surgical trauma results in persistent submaximal secretion of ACTH postoperatively, for the repeated administration of ACTH to normal adults results in an enhancement of adrenocortical responsiveness to ACTH (23).

Third, in 2 patients who had received long-acting ACTH preparations for the treatment of ulcerative colitis, a normal plasma 17-OHCS response occurred following total colectomy (8). This observation is of interest because, in man, both corticosteroid and corticotropin therapy have been shown to produce similar histologic alterations in the pituitary basophils (24). Although evidence exists that exogenous ACTH can partially inhibit the release of ACTH from the anterior pituitary of the rat (25), it would seem that major surgery may be a stress of adequate intensity to produce a normal pituitary-adrenal response in patients who have been treated with corticotropin. Experimental studies in dogs suggest that as little as 5 per cent of the anterior pituitary will provide sufficient corticotropin to maintain an adrenocortical response to stress (26).

In conclusion, it appears that patients who have been maintained with corticosteroid therapy prior to surgery may be expected to show a diminished adrenocortical response to operation. Consequently, they should receive supplementary adrenocortical steroid hormone therapy in preparation for surgery. However, patients who have received only corticotropin preparations prior to surgery may be expected to show a normal pituitary-adrenal cortical response to surgical trauma.

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JCEM

URINARY 17-KETOSTEROID AND 17-KETOGENIC STEROID EXCRETION IN CUSHING'S SYNDROME

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ABSTRACT

Utilizing Norymberski's method for determination of urinary 17-ketogenic steroid (glucocorticoid) excretion, normal subjects and patients with either suspected or proved Cushing's syndrome were studied with reference to their response to ACTH stimulation as an indication of adrenocortical function. Of 123 patients studied, 67 were found to have signs and symptoms suggestive of excessive adrenocortical activity. Sixteen of the 67 had definite signs and symptoms of Cushing's syndrome. Of the remaining 51 patients, 36 (70 per cent) showed abnormal responses to ACTH stimulation. The 16 patients with frank Cushing's syndrome were treated by bilateral adrenalectomy. It is postulated that Cushing's syndrome may initially be cyclic, or even self limited. A plea is made for continued and repeated observations of patients with signs and symptoms of early disease in order to ascertain the natural history, early treatment, and disability limitations of persistently abnormal adrenocortical function.

WITHIN the past few years we have seen a number of patients who exhibited many of the signs and symptoms associated with Cushing's syndrome. Although most of these patients did not show all of the findings of classic Cushing's syndrome, the clinical picture was consistent enough to suggest to us that the disease, perhaps milder in degree, did exist. This group is characterized clinically by a variety of vague complaints which include obesity, weakness, emotional instability, menstrual irregularities, hirsutism, and sometimes sterility. Frequently labile hypertension and early diabetes are found.

A preliminary study was made of a few of these patients. In some of them, during the control period, the 24-hour excretion of 17-hydroxycorticosteroids (17-OHCS) was elevated, as measured by the 17-ketogenic steroid method of Norymberski (1), and there was a marked rise following stimulation with ACTH.

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These preliminary observations indicated that a large number of patients fall into the foregoing clinical category, and that all degrees of Cushing's syndrome may exist. Since the laboratory findings in the few patients studied indicated an abnormal production of adrenocortical hormone, especially after ACTH stimulation, the study of urinary steroid excretion was extended to include a larger group of patients in the clinically borderline group. It was felt that such an approach might provide a means of earlier recognition of this condition and lend added clinical significance. Another large group of patients without any clinical evidence of adrenal dysfunction was also studied for control purposes.

METHODS AND MATERIALS

The 123 patients included in this study were divided into 3 groups on the basis of their presenting clinical picture. Group I consisted of 16 patients, 15 females and 1 male, with definite demonstrable clinical signs and symptoms compatible with Cushing's syndrome; their clinical findings are summarized in Table 1. Group II consisted of 51 patients, 47 females and 4 males, with symptoms suggestive of increased adreno-

TABLE 1. COMMON CLINICAL FINDINGS IN 16 PATIENTS WITH CUSHING'S SYNDROME

Degree	Number of patients with							
	Hypertension	Obesity	Edema	Osteoporosis	Weakness	Virilism	Menstrual disorders	Psychiatric disorders
Severe	6	9	5	2	16	11	12	10
Intermediate	8	6	9	13	0	4	1	6
None	2	1	2	1	0	1	3	0

TABLE 2. COMMON CLINICAL FINDINGS IN 51 PATIENTS WITH SUSPECTED CUSHING'S SYNDROME

Degree	Number of patients with							
	Hypertension	Obesity	Edema	Osteoporosis	Weakness	Virilism	Menstrual disorders	Psychiatric disorders
Severe	10	33	27	4	39	11	26	36
Intermediate	34	15	10	8	11	31	18	10
None	7	3	14	39	1	9	7	5

TABLE 3. COMPARISON OF URINARY STEROID EXCRETION IN 3 GROUPS OF SUBJECTS

Group	No. of subjects	17-Ketosteroids (mg./24 hrs.)		17-Ketogenic steroids (mg./24 hrs.)	
		Before ACTH	After ACTH	Before ACTH	After ACTH
I. *Cushing's syndrome*	16				
Average		13	43	37	159
Range		5–27	9–125	11–115	84–309
% Hyper-responsiveness or abnormally high values		25	19	56	100
II. *Suspected Cushing's syndrome*	51				
Average		10	29	19	79
Range		2–34	5–98	3–60	29–157
% Hyper-responsiveness or abnormally high values		16	26	11	70
III. a) *Control subjects*	21				
Average		10	24	13	41
Range		3–22	10–55	3–31	14–63
b) *Control subjects*	35				
Average		10		14	
Range		2–27		2–31	

cortical activity but in whom the clinical picture was borderline; their clinical findings are summarized in Table 2. Group II patients had most of the signs and symptoms of those in Group I but to a lesser degree. Group III consisted of 56 patients having no clinical evidence of increased adrenocortical activity; they were studied to serve as a control group.

In the 123 subjects, control 24-hour urinary 17-ketosteroid (17-KS) and 17-ketogenic (17-KGS) excretions were determined, using the Norymberski method. As shown in Table 3, in all patients of Groups I and II and in 21 patients of Group III (Group III-a), these studies were repeated following stimulation with ACTH; in 35 patients (Group III-b), only control urinary steroid values were determined (Table 3). In some patients plasma free 17-hydroxycorticoid (17-OHCS) levels before and after ACTH stimulation were determined, using the Porter-Silber method as described by Peterson, Karrer and Guerra (2).

The ACTH stimulation test was performed as follows: On the first day, a 24-hour (control) urine specimen was collected. The next day at 4 P.M., a dose of 80 units of Cortrophin-Zinc (Organon) was given intramuscularly. Sixteen hours later, at 8 A.M. on the third day, another 80-unit dose of Cortrophin-Zinc was given and collection of a 24-hour urine specimen was immediately started.

RESULTS

Controls

The control 24-hour excretion values for normal adults were 2–27 mg. for 17-ketosteroids and 2–31 mg. for 17-ketogenic steroids (Table 3, Groups III-a and III-b). In these normal groups, control values in females were lower (2–16 mg. for 17-KS and 2–28 mg. for 17-KGS) than in males (6–27 mg. for 17-KS and 4–31 mg. for 17-KGS). The response to ACTH stimulation in normal subjects was a rise of 150 to 350 per cent above normal values for 17-KS (an increment of 4 to 42 mg. per twenty-four hours) and a rise of 140 to 300 per cent for 17-KGS (an increment of 11 to 45 mg. per twenty-four hours).

Group I

All but 1 of the 16 patients who clinically exhibited frank Cushing's syndrome were females. All 16 patients were hyper-responsive to the ACTH test.

In 5 of the 16 patients, the control level of 17-KS excretion was elevated. With the ACTH test, 17-KS excretion rose in 4 of the 16 subjects, the range of increment being 9 to 65 mg. per twenty-four hours, a rise of 100 to 500 per cent over control values.

The 24-hour excretion of 17-KGS showed an absolute increase of 64 mg. or more (300 per cent or more of the control value). An absolute increase of 60 mg. and more than 300 per cent of the control value is considered to indicate hyper-responsiveness due to hyperfunction of the adrenal cortex. With the ACTH test, 4 of these 16 patients showed a response that was somewhat less than 300 per cent of the control value, but in these 4 cases the control excretions were elevated, and the absolute increment with ACTH was more than 64 mg. per twenty-four hours (64–194 mg., or a rise of 300–900 per cent over the control levels).

During the control period, plasma free 17-OHCS levels were determined in 7 of the 16 patients. In all 7 the levels were above the highest value in the normal range (20 μg. per 100 ml.).

These 16 patients were subjected to total adrenalectomy, with good results.

Group II

In the 51 patients with borderline Cushing's syndrome, the average control value for 24-hour 17-KS excretion was 10 mg., with a range of 2 to 34 mg. In 8 patients (16 per cent) the control values were abnormally high. Following stimulation with ACTH, the average 24-hour excretion of 17-KS was 29 mg., with a range of 5 to 98 mg. Thus the response to ACTH

was a 300–900 per cent rise above control levels, the absolute increment ranging from 3 to 70 mg. per twenty-four hours. In 13 patients (26 per cent), the response was abnormally high.

The excretion of 17-KGS in Group II was as follows: The average control value was 19 mg. per twenty-four hours, with a range of 3 to 60 mg. In 6 patients (11 per cent) the control values were high. Upon stimulation with ACTH, 17-KGS excretion rose on the average to 79 mg. per twenty-four hours, with a range of 29 to 157 mg. The increment with ACTH ranged from 19 to 125 mg., a rise of 300–900 per cent above control values. Thirty-six of these patients (70 per cent) exhibited an abnormally high 17-KGS response to ACTH.

Plasma free 17-OHCS levels were determined in 21 patients. In only 1 of them was the level in excess of the normal value of 20 μg. per 100 ml. before stimulation with ACTH.

DISCUSSION

In a classic case of Cushing's syndrome, there should be little difficulty in making the diagnosis. It is in the atypical or questionable case that diagnosis may present a problem, and frequently one must rely heavily on laboratory tests, particularly determinations of urinary steroid excretion.

Of the methods available for urinary steroid analysis, we found that of Norymberski to be the most adaptable and reproducible in our laboratory. Golub, Sobel and Henry (3) made a comparison of the Norymberski, Glenn-Nelson and Reddy methods of measuring urinary C_{21} steroids on the same urine sample and found the values to be consistently higher using the Norymberski method in both normal and in disease states associated with adrenal dysfunction. This was particularly true in patients with Cushing's syndrome, before and after stimulation with ACTH. We have found that the Norymberski method, although less specific, provides a more accurate estimate of 17-hydroxycorticoid secretion, particularly in suspected cases of Cushing's syndrome.

In the 16 cases of Cushing's syndrome (Group I), the correlation between clinical status and 17-ketogenic response to ACTH was quite good (Table 1; Table 3). In over half the cases there was a persistent elevation of the control 17-ketogenic steroid values, and all of these patients demonstrated hyper-responsiveness to ACTH. On this basis, about 25 per cent (16 to 67) of patients having signs and symptoms of Cushing's syndrome are in this category.

Much more common are the patients (51 of 67) with mild, variable signs and symptoms which tend to be ignored (Group II). However, a high incidence of adrenocortical hyper-responsiveness is found among them (Table 3). Of the 51 patients with suspected Cushing's syndrome, only 6

(11 per cent) showed elevated urinary 17-KGS excretion before ACTH, but 36 (70 per cent) showed elevated excretion after stimulation with ACTH. This demonstrates lability of function and definite hyper-responsiveness to ACTH. It may be that the adrenal cortex in cases of suspected Cushing's syndrome tends toward hyper-responsiveness only when subjected to stressful situations or direct stimulation with ACTH, but functions normally in the absence of these factors. At present, we cannot interpret these findings. There is no indication that the full syndrome ultimately develops in these patients. If that were true, many more cases of the classic syndrome should be found. On the other hand, the data may indicate that Cushing's syndrome is initially a cyclic disease with remissions and exacerbations dependent upon stressful situations (physical or emotional). However, if the duration and severity of stress exceeds a certain limit, the cyclic disease then may become a persistent one, in association with the typical clinical syndrome. The problem warrants further investigation before any conclusion can be drawn as to the exact status of patients in the Group II category. Close clinical observation is advisable in the hope that frank Cushing's syndrome can be detected and treated early. Certainly many patients with hypertension, diabetes, osteoporosis, oligomenorrhea, hirsutism or obesity deserve to be studied as described here. When two or more of these abnormalities are present concurrently, the urgency of such evaluation of adrenocortical activity is even greater.

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THE 17-HYDROXYCORTICOSTEROIDS OF PAROTID FLUID, SERUM AND URINE FOLLOWING INTRAMUSCULAR ADMINISTRATION OF REPOSITORY CORTICOTROPIN*

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ABSTRACT

In healthy young adult males, the intramuscular injection of 40 I.U. of corticotropin produced the expected elevations in the levels of serum and urinary 17-hydroxycorticosteroids (17-OHCS). The level of 17-OHCS in parotid-gland fluid closely paralleled that in the serum. These data strengthen the premise that the level of free 17-OHCS in parotid-gland fluid may well be utilized in evaluating adrenocortical status. The use of this method permits continuous sampling of a completely nonstressful nature and, even in extremely close quarters where blood sampling is impossible, the subject can easily obtain specimens of his own parotid fluid.

PREVIOUS communications from these laboratories (1, 2) pointed out that the oral administration of single 50-mg. doses of hydrocortisone, Δ^1-hydrocortisone (prednisolone), 6-methyl-Δ^1-hydrocortisone (Medrol), or Δ^1,9α-fluoro-16-hydroxyhydrocortisone (triamcinolone) produced parallel changes in the levels of free 17-hydroxycorticosteroids (17-OHCS) in serum and parotid fluid and that changes in the excretion of total 17-OHCS follow this pattern closely. Based upon these findings, it was suggested that the level of free 17-OHCS in parotid fluid might possibly be used as a sensitive indicator of adrenocortical status. To test this premise, the present study in which adrenocortical stimulation was produced by injection of corticotropin (ACTH) was designed.

PROCEDURE AND METHODS

A group of 28 male subjects between 17 and 22 years of age was studied; 18 were given ACTH, and 10 were used as controls. All participants had been proved free of disease by recent medical examination, and each was judged physically qualified for military service. Such factors as diet, environmental exposure and times of arising and

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* The opinions asserted are those of the authors and are not to be construed as representing the views of the United States Air Force.

retiring were virtually identical in all instances. Immediately prior to 9:00 A.M., samples of blood and parotid fluid were collected and each subject force-voided urine. Eighteen of the subjects received 40 U.S.P. units of Corticotropin Gel (Wilson Laboratories) by intramuscular injection at 9:00 A.M. (time zero). Collections of blood and parotid fluid were repeated at two, four and six hours after time zero, and a single urine sample was collected from each subject over the entire six-hour study period. Parotid fluid was collected in graduated centrifuge tubes under the stimulation of sugared chewing gum,[1] the 3-gram bolus being changed every five minutes in order to maintain the desired rate of flow. In all instances, a ten-minute accommodation period (waste sample) was allowed prior to the collection of the sample for analysis. The levels of 17-OHCS in serum and parotid fluid were determined by the method of Peterson *et al.* (3) and total 17-OHCS levels in urine were determined by a modification combining the methods of Reddy (4) and Clayson (5).

RESULTS AND DISCUSSION

The effect of intravenous and intramuscular injection of ACTH on 17-OHCS levels in blood is well known (6–10), and it is generally agreed that the functional status of the adrenal cortex is dependent upon the circulating level of corticotropin. In the present study, the mean serum concentration of free 17-OHCS in the 18 ACTH-treated subjects rose from 13 μg. per 100 ml. before injection to 35 μg. per 100 ml. two hours after receiving ACTH (Table 1). These findings agree closely with those of past work (6). The mean parotid-fluid concentration of free 17-OHCS in the same subject increased from 4.1 μg. to 10.3 μg per 100 ml. over the same time-period; no subject failed to show an increase. The percentage increase in 17-OHCS concentration, based upon the mean values at time zero, was virtually identical for the two fluids.

Following the maximal rise in the serum steroid level at two hours, a a relatively high concentration was found during the remainder of the study. After six hours the level was still 27 μg. per 100 ml.—approximately double the mean value at time zero. For parotid fluid, however, the downward trend was more pronounced, the level at six hours being only 5.5 μg. per 100 ml. This divergence suggests that some process more complicated than simple diffusion must be involved in the passage of steroid from the blood to the parotid fluid. The comparative levels two hours after administration of ACTH, if taken alone, suggest that simple diffusion might account for the change of steroid concentration in the parotid fluid. Moreover, simple diffusion is suggested by our unpublished findings that the steroid concentration in parotid fluid seems to be independent of rate of flow. The possibility remains, however, that the drop in the parotid-fluid level of 17-OHCS without a proportionate drop in the serum level was due to a more complex process, such as exhaustion of the glandular response

[1] Furnished by Mr. Robert Heggie, Vice-President, American Chicle Company.

TABLE 1. FREE 17-OHCS LEVELS IN SERUM AND PAROTID FLUID FOLLOWING ADMINISTRATION OF ACTH

Groups	Free 17-OHCS level (μg./100 ml.)							
	Hours after ACTH							
	0		2		4		6	
	Serum	Parotid fluid	Serum	Parotid fluid	Serum	Parotid fluid	Serum	Parotid fluid
ACTH, 40 I.U. intramusc. (18 subjects)	13.7* ±3.6	4.1 ±1.6	35.1 ±4.3	10.3 ±2.1	33.7 ±7.7	7.4 ±2.3	27.0 ±10.1	5.5 ±1.7
Controls; no treatment (10 subjects)	15.0 ±3.8	4.1 ±1.4	14.0 ±3.4	5.1 ±1.3	13.9 ±4.0	3.9 ±1.0	12.7 ±5.0	3.7 ±1.6

* Mean and standard deviation.

following prolonged elevation of 17-OHCS concentration in the serum. Further studies with: (a) intravenous administration of ACTH, thus assuring equal stimulation of the target cells and a relatively constant time-response between subjects, and (b) isotopic hydrocortisone, should clarify this problem.

The control subjects maintained relatively constant serum steroid levels throughout the study. A downward trend, particularly in the six-hour sample, was in agreement with the reported diurnal pattern for serum 17-OHCS (11). Although the level of 17-OHCS in the parotid fluid was lower at the close of the study than at the beginning, a systematic decrease during the day was not found.

The rates of flow of parotid-gland fluid in the ACTH-treated subjects and in the controls were remarkably similar at each of the four times of sampling. For the ACTH-treated subjects the mean flow rates at these times were .73, .71, .74 and .70 ml. per minute (s.d., .21, .23, .25 and .22). For the controls, the corresponding mean values were .66, .67, .73 and .68 ml. per minute (s.d., .23, .26, .22 and .26). The mean excretion rate of total 17-OHCS in urine for the ACTH-treated subjects was .95 mg. per hour (s.d., .26), and that for the controls was .46 mg. per hour (s.d., .17).

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ACUTE FATAL PARATHYROID POISONING ASSOCIATED WITH NECROSIS OF THE PARATHYROID ADENOMA PRIOR TO DEATH

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ABSTRACT

A case of acute fatal parathyroid poisoning is reported. The patient had an extremely high level of serum phosphorus and a normal level of serum calcium. Autopsy revealed heavy deposits of calcium in all the internal organs, as has been previously seen in parathyroid poisoning. The parathyroid adenoma showed diffuse necrosis with thrombosis of almost all the vessels. The probable reason for the hyperphosphatemia is discussed.

HYPERPARATHYROIDISM can cause parathyroid poisoning if the serum calcium level exceeds the critical value of 20 mg. per 100 ml. Grollman (1) has shown that the filtrability of serum inorganic phosphorus is dependent upon ionic calcium concentration in various species. Howard *et al.* (2) have demonstrated that phosphate is no longer completely filtrable if the serum calcium level is about 20 mg. per 100 ml. The hypercalcemia of severe hyperparathyroidism may cause hyperphosphatemia and, in association with a rapidly rising level of nonprotein nitrogen, end in "chemical death" (3).

So far as is known, only 7 cases of fatal parathyroid poisoning have been reported in the literature (4–8, 13, 14). In addition, Lowenburg and Ginsburg (15) reported the case of a 5-year-old boy who had received by mistake large doses of calcium gluconate and 100 units of parathyroid hormone for a period of six days; he showed the clinical symptoms of parathyroid poisoning. Howard *et al.* (9) observed parathyroid poisoning of short duration prior to necrosis of the adenoma and spontaneous remission in a case of hyperparathyroidism. Collip and Clark (10, 11) produced "chemical death" in dogs by administration of parathyroid extract as well as infusions of calcium chloride and sodium dihydrogen phosphate.

The purpose of this paper is to report the following case of acute fatal parathyroid poisoning:

CASE REPORT

Ch. W. (Med. Kl. 1327/58) was a 55-year-old physician who was admitted to the hospital on April 7, 1958. He had had severe epigastic pain as well as aching in the

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knees and legs for fourteen days, but had been able to work until five days prior to hospitalization. The patient had frequently vomited and had been somnolent for three days before entry. He had been admitted to another hospital for treatment of duodenal ulcer in May 1956. The x-ray films made because of his ulcer showed the presence of moderate osteoporosis. He had had polydipsia for one year. There was no history suggestive of a renal disorder.

Physical examination: The patient had a red-tinged, cyanosed face. The liver was slightly enlarged, and there was a moderate degree of dermatomycosis. He was disoriented but conscious and responded to various stimuli such as pinching and shouting. There was no hyporeflexia. The body temperature was 36.6 C., pulse 110 per minute, and blood pressure 140/110 mm. Hg.

An electrocardiogram showed sinus tachycarida, T-S segment depression in I and II, shallow inversion of the T wave in I and II, depression of T-S segments in V_3-V_8 and shallow inversion of T waves in the same leads. There was no change in the QRS complex.

The erythrocyte sedimentation rate was 61 mm. in the first hour. The hemoglobin level was 16.9 Gm. per cent, red blood cell count 5,400,000 per cu. mm., leukocyte count 15,500 with a moderate shift to the left, and thrombocyte count 120,000 per cu.mm. There were no cells in the cerebrospinal fluid and the values for glucose, protein and chloride concentration were normal.

The serum level of sodium was 132 mEq., potassium 3.3 mEq., and chloride 105 mEq. per liter. CO_2 combining power was slightly increased and the blood pH was 7.4. The serum level of calcium was 10.6 mg., phosphorus 25.3 mg., and nonprotein nitrogen 108 and 114 mg. per 100 ml. on the first and the second day respectively. The 24-hour volume of urine was 1780 ml. on the first day and 1010 ml. on the second day; specific gravity was 1.011 on the two days. There were red and white blood cells and a trace of albumin in the urine.

The patient became unconsious on the second day and died in a state of collapse on the third day of hospitalization.

Precomatous uremia was probably present, though there had been no sign of previous renal disorder. The high level of serum phosphorus with no acidosis, the polydipsia, the duodenal ulcer and the osteoporosis known for two years were, however, highly suggestive of parathyroid poisoning, even though the serum calcium level was normal in the acute state.

It was assumed that severe hyperparathyroidism with a very high serum calcium level might have caused the hyperphosphatemia. A sudden lack of parathyroid secretion had then resulted in a fall in the concentration of serum calcium. Thus the phosphate had again become completely filtrable, but had been reabsorbed in the renal tubules to an increased extent because of the relative lack of parathyroid hormone.

Autopsy revealed osteitis fibrosa generalisata with calcium deposits in all the internal organs, notably the wall of the heart, the alveolar membrane of the lungs, the mucosa of the stomach and the basal membrane of the renal tubules. Many of the glomeruli were hyalinized and showed calcium deposits (Figs. 1 and 2). The parathyroid adenoma was approximately 1 cm. in length. The microscopic sections showed large areas of necrotic tissue with thrombosis of almost all the vessels (Fig. 3). The remaining parathyroid tissue seemed to be normal in the microscopic sections.

In several of the cases of parathyroid poisoning reported in the literature, the levels of both serum calcium and serum phosphate were elevated.

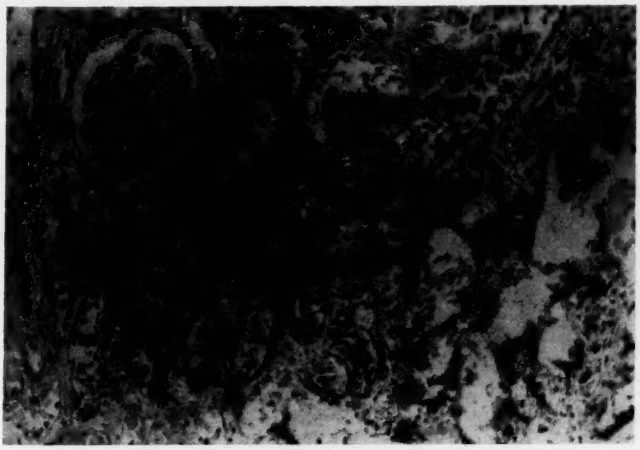

FIG. 1. Calcium deposits in the basal membrane of the renal tubules (Von Kossa's silver stain).

Collip and Clark (10, 11) reported without explanation a rise in the serum phosphorus level in spite of a fall in the serum calcium level in a few dogs of a series of animals subjected to parathyroid poisoning; it is possible that some of the parathyroid extracts given in the last phase were inactive. Howard *et al.* (9) described a case of hyperparathyroidism with spontaneous remission due to acute necrosis of the parathyroid adenoma. A case of hemorrhage of the parathyroid gland resulting in tetany was observed

FIG. 2. Calcium deposition in the wall of the heart (Von Kossa's silver stain).

FIG. 3. Parathyroid adenoma with large areas of necrosis and thrombosis of the blood vessels.

by Norris (12). The present case could be an instance of spontaneous remission in hyperparathyroidism, in which calcium deposition in the course of hypercalcemia and hyperphosphatemia was too severe to be survived.

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Letters to the Editor

ALDOSTERONE EXCRETION IN TWO ADRENALECTOMIZED PREGNANT WOMEN

To the Editor:

The adrenalectomized pregnant woman offers an opportunity to examine the role of the placenta in the production of aldosterone. In normal pregnancy the excretion of aldosterone and its reduced metabolite is significantly increased (1–4), whereas in adrenalectomized pregnant women and those with adrenal insufficiency the excretion is low or negligible. Laidlaw *et al.* (5) reported the findings in 2 pregnant women; in one, during the nineteenth week of gestation, the 24-hour excretion of aldosterone was 1.3 μg.; in the other, during the thirty-eighth week, the excretion was 4.4 μg. During pregnancy in a woman with Addison's disease, Beaulieu *et al.* (6) could detect no urinary aldosterone, and Christy and Jailer (7) found only insignificant amounts in the urine of a similar patient.

The following studies on 2 totally adrenalectomized pregnant women offer additional information on the excretion of aldosterone and other steroids during gestation. Aldosterone excretion of the 2 newborn infants was also measured.

CLINICAL MATERIAL AND METHODS

Patient A.O. In 1954 a hyperplastic left adrenal was completely removed because of Cushing's disease. Nevertheless, the disease progressed, and eleven months later a right total adrenalectomy was performed. The patient was maintained with 37.5 mg. of cortisone per day, and two years later she became pregnant. She continued to receive the same amount of cortisone daily in fractional doses, the last dose being given at 10 P.M. on the day prior to the collection of urine. On the day of collection she received 0.75 mg. of 9α-fluorocortisol only.

Patient H.M. This patient had Cushing's disease. A complete left adrenalectomy was followed one month later by a complete right adrenalectomy. Approximately three months later she became pregnant. During the periods of urine collection she continued to receive 40 mg. of cortisol and 0.25 mg. of 9α-fluorocortisol daily.

Methods. The aldosterone fraction obtained after hydrolysis at pH 1 was purified in two chromatographic systems (the toluene-propylene glycol system of Zaffaroni and the B_5 system of Bush) and was assayed biologically by the method of Venning *et al.* (8).

Urinary pregnanediol and pregnane-3α,17α,20α-triol (pregnanetriol) were measured by a modification of the method of Bongiovanni and Clayton (9) following hydrolysis with β-glucuronidase. Total 17-hydroxycorticosteroids were determined by the Silber-Porter method (10). For the measurement of individual 17-hydroxycorticosteroids—cortisol (compound F), cortisone (compound E), tetrahydrocortisol (THF) and tetrahydrocortisone (THE)—the urine was incubated with β-glucuronidase for forty-eight hours at pH 5, then acidified to pH 1, and immediately subjected to extraction. The dried neutral fraction was run twice in the chloroform-formamide system. Cortisone and material more polar than this steroid were applied on a paper strip and were separated in the toluene-propylene glycol system. Characterization studies by sulfuric-acid chromogen spectra showed that the blue-tetrazolium reactive steroids having polarities similar to those of THF and THE had spectrum patterns similar to those of standard THF and THE. The material from the cortisone zone separated into 3 spots upon reapplication in the chloroform-formamide system, and the fraction measured had a sulfuric-acid chromogen spectrum similar to that of authentic cortisone. These 17-hydroxycorticosteroids were determined separately by means of ultraviolet absorption, Porter-Silber reaction and reduction with blue tetrazolium. No measurements were made of the metabolites of 9α-fluorocortisol.

RESULTS

The results are shown in Tables 1 and 2. The values are expressed per gram of creatinine. Both patients excreted normal amounts of pregnanediol and pregnanetriol. The latter finding is in agreement with observations of Herrman and Silverman (11) in their study of a bilaterally adrenalectomized patient. It is possible that the ovaries or fetal adrenals may have hydroxylated small amounts of progesterone at C_{17}, which were then metabolized to pregnanetriol. Four days following delivery, no pregnanetriol was found in the urine of Patient A. O.

In Patient A. O. only a trace of aldosterone could be detected in the urine either ante partum or post partum. In Patient H.M. the excretion ranged from 10.1 to 13.8 μg. per Gm. of creatinine. Following delivery, on two occasions, small amounts of aldosterone could be detected. The newborn infants excreted 1.4 and 2.5 μg. per twenty-four hours on the third and sixth days, respectively.

The source of the aldosterone excreted by Patient H.M. is puzzling. The possibility that the placenta may be capable of forming this mineralocorticoid seems unlikely, as incubation studies carried out by Sybulski (12) did not demonstrate any synthesis of aldosterone in this tissue. Similar conclusions have been reported by Christy and Jailer (7). There was no clinical indication that adrenocortical tissue had regenerated in Patient H.M., although this has occurred in a patient in our clinic who was supposedly totally adrenalectomized. Small amounts of aldosterone have been detected chemically by Laidlaw *et al.* (5) in the urine of an

TABLE 1. STEROID EXCRETION* IN 2 ADRENALECTOMIZED PREGNANT WOMEN

	Therapy	Pregnanediol (mg.)	Pregnanetriol (mg.)	Aldosterone (μg.)	P-S corticosteroids (mg.)	17-Ketosteroids (mg.)
Patient A.O.†						
1956						
Oct. 9	0.75 mg. 9α-Fl.F	26.0*	1.2	<1	1.65	3.5
Dec. 5	0.75 mg. 9α-Fl.F	66.5	9.7	<1	0.87	4.1
1957						
Jan. 23	0.75 mg. 9α-Fl.F	62.0	1.9	<1	1.93	5.7
Feb. 3	*Delivery*					
Feb. 7	0.75 mg. 9α-Fl.F	2.9	0	<1	0.47	2.5
Patient H.M.‡						
1956						
July 10	40 mg. F					
	0.25 mg. 9α-Fl.F	95.0	—	10.1	—	—
Sept. 5	40 mg. F					
	0.25 mg. 9α-Fl.F	35.0	3.5	13.8	3.9	—
Sept. 23	*Delivery*					
Sept. 24	90 mg. F					
	0.5 mg. 9α-Fl.F	—	—	2.8	—	—
Sept. 29	30 mg. F					
	0.25 mg. 9α-Fl.F	—	—	2.3	—	—
Baby O		—	—	1.4§	—	—
Baby M		—	—	2.5§	—	—

* Results expressed per Gm. of creatinine.

† Adrenalectomized in 1954. Maintenance dose of cortisone (37.5 mg.) not given on day of collection of urine.

‡ Adrenalectomized in 1955.

§ Results expressed as μg. per 24 hours.

adrenalectomized pregnant woman. The amounts found in the urine of her newborn infant were too small to account for the excretion of aldosterone in the urine of Patient H.M. The relative amounts of free cortisone, cortisol, THE and THF are shown in Table 2; included are values obtained in a similar manner on a normal pregnant woman and a normal nonpregnant woman. As Patient A.O. was receiving cortisone up until 10 P.M. on the day preceding the collection of urine, and Patient H.M. continued to receive cortisol during the collection period, the relatively different amounts of compound E and compound F in the urine of these patients may be partly explained on this basis. Both of these adrenalectomized

TABLE 2. EXCRETION* OF CORTISOL (F), CORTISONE (E), AND THEIR REDUCED METABOLITES IN ADRENALECTOMIZED PREGNANT WOMEN VERSUS NORMAL PREGNANT AND NONPREGNANT WOMEN

Patient	Condition	F (mg.)	E (mg.)	THF (mg.)	THE (mg.)	Ratio: $\frac{THF+THE}{F+E}$
H.M.	Adrenalectomized pregnant, 9 mos.	0.36	0.27	0.68	0.59	2.02
E.O.	Adrenalectomized pregnant, 6 mos.	0.09	0.79	0.12	0.39	0.58
	8 mos.	0.07	0.39	0.03	0.25	0.61
	9 mos.	0.16	0.65	0.11	0.46	0.70
	4 days post partum	0.07	0.28	0.05	0.14	0.70
E.W.	Normal pregnant, 8 mos.	0.19	0.40	0.42	1.99	4.08
M.E.	Normal not pregnant	0.16	0.17	0.75	1.96	8.10

* Results expressed per Gm. of creatinine.

patients failed to metabolize the administered steroid to the reduced compounds THE and THF in the ratio usually observed in normal pregnancy. The lower ratio of reduced metabolites to free urinary corticosteroids in pregnancy is in accord with recent observations of a reduced clearance of administered cortisol as well as of endogenous corticosteroids during gestation (13–15). This slower rate of catabolism of cortisol may in part be associated with higher levels of circulating estrogens in pregnancy, as it has been shown that administered estrogens will delay the rate of clearance of cortisol in the nonpregnant subject (16, 17).

Although Patient A.O. was maintained with a constant amount of cortisone, total urinary corticosteroids and 17-ketosteroids decreased following delivery.

SUMMARY

Steroid excretion has been measured in 2 totally adrenalectomized pregnant women. Normal amounts of pregnanediol and pregnanetriol were observed. One of the patients excreted only a trace of aldosterone, whereas the other excreted from 10.1 to 13.8 μg. per Gm. of creatinine. The possibility that regeneration of adrenal tissue occurred in the latter

case cannot be excluded, although it was not indicated by the clinical condition.

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Addendum

Since completing this study, the response to the intravenous administration of 25 units of ACTH was investigated in Patient H.M. A dose of 2 mg. of 9α-fluorocortisol was given daily throughout the test. The control urinary values for 17-hydroxycorticosteroids were 2.7 mg. and 3.6 mg., and for 17-ketosteroids 6.8 mg. and 4.8 mg. per twenty-four hours. During administration of ACTH, the 17-hydroxycorticosteroids were 4.9 mg. and 3.6 mg., and the 17-ketosteroids 5.6 mg. and 5.7 mg. per twenty-four hours. Although the values for 17-hydroxycorticosteroids were slightly high for an adrenalectomized patient, there was no response to ACTH.

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THE RATE OF DISAPPEARANCE OF ACTH ACTIVITY FROM THE BLOOD OF HUMANS

TO THE EDITOR:

It is well known that in man and animals, a given amount of ACTH administered continuously over a period of time produces a greater biologic effect than the same amount of ACTH administered as single or intermittent doses (1–4). Furthermore, in the hypophysectomized dog (steroid content of adrenal venous blood) the measurable steroid secretory effect of submaximal doses of intravenous ACTH (up to 10 milliunits) is complete in fifteen to thirty minutes (5). Such observations suggest that ACTH is either excreted or inactivated promptly.

That ACTH can be inactivated by plasma (6, 7) or blood (7, 8) has been reported, but has been denied by others (9, 10) for rat ACTH in rat

plasma. It has been demonstrated that ACTH is "bound" to kidney and adrenal tissue (9), but whether this binding phenomenon is an important facet in the catabolism of ACTH is not known. Blood or tissue inactivation and binding could explain the relatively short "half-life" of ACTH activity in the intravascular compartment as observed in animals with the use of endogenous ACTH (10, 11), exogenous species-specific ACTH (9, 12), and exogenous heterologous ACTH (13, 14).

Only one estimation of the duration of ACTH (hog) activity in the blood compartment of man has been reported (15). Therefore, it was felt that the following observations on the "half-life" of ACTH (hog) in human subjects, made incidentally to other studies (16), were of interest.

METHODS

The general methods employed were as follows. One of 2 commonly used commercial hog ACTH preparations (designated A or B) of equivalent U.S.P. rated potency were given intravenously in 0.9 per cent (w/v) sodium chloride or 5 per cent (w/v) glucose over periods of two or eight hours in doses of 25 or 50 U.S.P. units. Venous blood samples (20 to 80 ml.) were drawn (through a 19-gauge needle into a syringe containing heparin) over a period of one minute, at 0 to 45 minutes after the end of the infusion. The plasma was separated and placed in a freezing compartment within thirty minutes of blood withdrawal. The ACTH activities of duplicate plasma samples were bioassayed within one to two weeks by the hypophysectomized dog method (5, 17).

RESULTS AND DISCUSSION

When the logarithm of ACTH activity (milliunits per 100 milliliters of plasma) was plotted against time it appeared that there were 2 rates of fall in activity, *viz*, a rapid first phase and a slower second phase, as illustrated by the data for Subjects M.M. and T.A. in Table 1. The data for the second phase were not considered sufficiently complete to calculate "half-lives."

The faster initial phase could represent the rapid distribution of ACTH throughout its "hormone space" superimposed upon the slower rate of excretion and/or inactivation reflected by the second phase. The range for the first phase "half-lives" was 4 to 18 minutes. These data are too few to indicate any differences based on the type of patient or the type of ACTH.

The slower second phase appeared definitely only in Subjects M.M. and T.A. who were given 50 units of ACTH "A" over a period of eight hours. If larger doses of ACTH had been given or if a more sensitive method of ACTH assay had been available, the second phase could have been more clearly defined.

It may also be seen from Table 1 that the initial zero levels of ACTH

TABLE 1. DISAPPEARANCE OF EXOGENOUS ACTH FROM THE PLASMA OF HUMAN SUBJECTS

Subject	Intravenous infusion of hog ACTH			ACTH activity (milliunits/100 ml. plasma)* at time-intervals after infusion							Half-life (minutes), 1st phase	Remarks
	Type	Dose U.S.P. (units)	Duration of infusion (hrs.)	0	10 mins.	15 mins.	20 mins.	25 mins.	30 mins.	45 mins.		
R.D.	A	25	2	86	14		12	8			4	Normal
E.R.	A	25	2	112		23			0		6	Cushing's syn.
G.N.	A	25	2	87		40			6		8	Hypothyroidism
E.M.	A	25	8	36		13			0		11	Addison's dis.
W.D.	A	25	8	38		9			4†		9	Addison's dis.
J.S.	A	25	8	57		16			0		9	Addison's dis.
J.J.	A	25	8	44	15		8			6	6	Normal
M.M.	A	50	8	114	67			52		64	18	Normal
T.A.	A	50	8	104	48		20			16	9	Normal
N.H.	B	50	8	15	10		7		6		18	Normal
N.M.	B	50	8	10	6		4†		3†		14	Normal
B.B.	B	50	8	14	7			5†		3†	10	Normal

* All data represent the mean of duplicate determinations.

† Borderline validity; the plasma concentration of ACTH (milliunits per 100 ml.) was at the lower limit of the assay sensitivity.

activity were always greater for ACTH "A" and that the activity of ACTH "A" appeared to persist for a longer period of time than the activity of ACTH "B." The differences could be due to a difference in the steroidogenic potency of the two preparations or to a difference in the "half-life" of ACTH activity in the plasma compartment. Subsequent work in the human, dog and rat has shown that ACTH "A" is much more steroidogenic than ACTH "B" (16).

CONCLUSIONS

It appears reasonable to conclude that hog ACTH administered intravenously to human subjects disappears from this compartment relatively quickly. This rapid disappearance accounts for the greater total effect of a given dose when administered slowly in a continuous manner compared with the effect of the same total dose given quickly. The rapid disappearance (and apparent inactivation) of ACTH from the blood stream is probably one of the factors in the maintenance of hypothalamic-pituitary-adrenal activity within the relatively narrow limits observed in the healthy unstressed human.

The ultimate fate of intravascular ACTH is still not known. Further studies with shorter time-intervals between blood samplings and the use of more purified ACTH preparations are necessary in order to determine the

"half-life" of intravascular ACTH with precision for both heterologous and human ACTH when available.

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LACK OF DIURNAL VARIATION IN THE LEVEL OF SERUM PROTEIN-BOUND IODINE

TO THE EDITOR:

The purpose of this letter is to describe the fluctuations of the serum protein-bound iodine (PBI) level in euthyroid subjects during the day and night. The concentration of serum PBI in euthyroid women has been shown to fluctuate within a narrow range, the highest levels occurring during the luteal phase of the menstrual cycle; fluctuation (not obviously cyclic) was also noted in 2 male subjects (1). Volpé *et al.* (unpublished data) found that in healthy euthyroid adults, from test to test, there is a fluctuation of the serum PBI level, which is slightly greater than the range of technical error. The blood samples were taken at any convenient time during the day; the question arose as to whether the concentration of serum protein-bound iodine was influenced by the time at which the samples were drawn. This same problem would theoretically apply to any random specimen of blood obtained for PBI determination.

To date, the amount of data accumulated regarding diurnal variations in the serum PBI level is scant. Tingley *et al.* (2) observed no consistent fluctuation in euthyroid young adults between the hours of 7 A.M. and 5 P.M.; however, blood samples were not obtained after 5 P.M.

Since 1956, it has been known that the concentration of plasma hydroxycorticoids in normal human adults shows a diurnal variation (3). Our study was carried out to determine whether there was a similar significant daily fluctuation in the concentration of serum PBI in euthyroid subjects.

MATERIALS AND METHODS

Seven patients were selected—4 males and 3 females; their ages ranged from 17 years to 80 years, with a mean age of 52.3 years. The patients were all euthyroid and were in the hospital undergoing treatment for a variety of illnesses (see Table 1). None of them had a history of thyroid disease, nor had been treated with antithyroid medication. They had not received any drugs and had not been exposed to any materials that were known to interfere with the serum PBI determination.

Blood samples were drawn at 8:00 A.M., 1:30 P.M., 5:30 P.M. and 3:00 A.M. These times were selected arbitrarily with no special relation to meals or activity, except that no patient was subjected to more exertion than the normal ward routine.

Serum PBI values were determined by a modification of the method of Barker, Humphrey and Soley (4), and all analyses were made in duplicate. For euthyroid adults our laboratory has established the range of PBI values as 3.6 to 6.5 μg. per 100 ml. of serum.

RESULTS

Our observations showed insignificant fluctuations throughout the day which did not conform to any particular pattern. The results of the PBI determinations for each subject are presented in Table 1.

DISCUSSION

Tingley *et al.* noted in euthyroid medical students that the stress of oral examinations caused a significant elevation in the serum PBI level

TABLE 1. THE SERUM PBI LEVEL IN 7 EUTHYROID PATIENTS AT VARIOUS TIMES THROUGHOUT THE DAY AND NIGHT

Patient	Sex and age (yrs.)	Disease	Serum PBI level (μg./100 ml.)*			
			8:00 A.M.	1:30 P.M.	5:30 P.M.	3:00 A.M.
L.C.	M 49	Pulmonary fibrosis of unknown etiology. Anxiety neurosis	4.39	4.53	4.61	4.12
A.B.	M 72	Diabetes mellitus	5.36	5.36	5.85	5.85
S.G.	M 63	Pruritis ani	5.31	5.40	5.06	5.61
G.W.	M 19	Tetralogy of Fallot	4.12	4.30	4.21	4.06
S.H.	F 80	Cardiovascular accident. Hypertensive heart disease	5.42	5.36	5.43	5.43
E.P.	F 17	Chronic discoid lupus	5.79	5.98	5.23	5.20
L.W.	F 66	Myocardial infarct. Diabetes mellitus	5.37	5.54	4.56	5.16

* Average of duplicate determinations on each specimen.

The standard deviation for duplicate determinations in this series was 0.36 μg., which is in accord with previous results obtained in our laboratory. An analysis of variance revealed no significant fluctuation in the serum PBI concentration of the 4 blood samples obtained from each patient.

in blood samples taken following the examinations (2). However, this observation conflicts with the data of Volpé *et al.*, who found that certain acute stresses had no influence on the serum PBI level in euthyroid persons (unpublished data). The patients of our present study were euthyroid and gave no history of previous thyroid disease; nevertheless they were all ill, convalescing from a variety of disorders. No patient had been exposed to any harassing situation for at least forty-eight hours prior to the study.

The results of this study indicated that there was no significant diurnal variation in the serum PBI level of these 7 euthyroid adults.

Acknowledgments

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ADDISON'S DISEASE COMPLICATED BY GRAVES' DISEASE

TO THE EDITOR:

Hyperthyroidism occurring during the course of Addison's disease is so rare that it seemed worth while to report the following case:

CASE REPORT

A 44-year-old farmer was admitted to Medical Department A of Oslo University Hospital in January 1959. His illness dated back to 1950, when a diagnosis of Addison's

disease had been made from a history of weight loss, fatigability and muscle stiffness and from the findings of low blood pressure and pigmentation of the skin and oral mucous membrane. Results of a tuberculin skin test were negative. X-ray examination of the chest showed an old pleural lesion on the right side. X-ray examination of the adrenal region did not reveal any calcification. A glucose tolerance curve was normal. No steroid analyses were performed.

Between 1951 and 1958 the patient had been admitted four times to Medical Department A. Except for the one time when he was admitted in a state of impending Addisonian crisis, the disease had been well controlled with 12.5–25.0 mg. of cortisone daily, implantation of pellets of desoxycorticosterone acetate (DCA), and oral administration of salt. During all these previous admissions the thyroid gland had been normal on palpation.

At the time of the fifth admission in January 1959 he stated that although he had felt well most of the time since the implantation of DCA one year previously, he had lost some weight during the last few weeks, had felt weak, and craved salt. On physical examination, a striking change was noted in his appearance. The palpebral fissures were wide and there was bilateral exophthalmos with periorbital edema. The exophthalmos measured 21 mm. and 22 mm. (Hertel). There was no chemosis or ocular palsy. The patient had noted no heat intolerance, diarrhea, palpitation, nervousness, tremor, change in the texture of his skin or hair, or tenderness of the neck.

Blood pressure was 130/70 mm. Hg and the pulse was regular with a rate of 90 per minute. The thyroid gland was diffusely enlarged; the weight was estimated to be 30 Gm. The gland was firmer than normal, but no nodules were felt. There was no bruit and the gland was not tender.

The urine was normal. The hemoglobin level was 14 Gm. per 100 ml., red blood cell count 5,700,000 per cu. mm., and white blood cell count 10,000 with 9 per cent eosinophils, 37 per cent polymorphonuclear cells, 50 per cent lymphocytes and 4 per cent monocytes. The eosinophil count was 519 per cu. mm. The level of blood urea was 42 mg. per 100 ml., serum cholesterol 245 mg. per 100 ml., total base 150 mEq. per liter, chlorides 102 mEq. per liter, and potassium 4.8 mEq. per liter. The skull and trachea were normal by x-ray examination. Basal metabolic rates on two occasions were +109 per cent and +123 per cent.

During the stay in the hospital, the diagnosis of Addison's disease was confirmed by means of an ACTH stimulation test. The results of radioiodine studies are shown in Table 1.

COMMENT

This case illustrates the occurrence of Graves' disease during the course of chronic adrenal insufficiency.

According to Rupp and Paschkis (1), only 8 well documented cases of co-existing Addison's disease and thyrotoxicosis had been reported up to 1957. Since then, 1 more case has been described in addition to the present one (2). In spite of the small number of cases, Fredrickson (3) states that there is a greater incidence of hyperthyroidism in Addison's disease than in normal persons. If this be true, one might visualize the presence of a pituitary mechanism, with the hypercorticotropinism of Addison's disease leading to hyperthyrotropinism and secondary hyperthyroidism. In that

TABLE 1

	Thyroidal uptake of I^{131} (% dose)			
	1 hr.	2 hrs.	3 hrs.	24 hrs.
Control	22	31	42	59
After 0.16 mg. of triiodothyronine daily for 7 days	21	34	—	51
After 1 mg. of 9α-fluorohydrocortisone daily for 7 days	14	25	32	55

instance, it should be possible, by means of the thyroidal I^{131} uptake test, to demonstrate suppression of the pituitary by adrenocortical and thyroid hormones. The findings reported here do not support this hypothesis. Failure to suppress the increased thyroidal uptake of I^{131} by administration of triiodothyronine (Table 1) indicates the presence of genuine Graves' disease. The lack of effect of 9α-fluorohydrocortisone on thyroidal I^{131} uptake (Table 1) suggests that there is no connection between the increased secretion of ACTH in Addison's disease and the development of thyrotoxicosis. Admittedly, this suggestion would be considerably stronger had one of the newer, powerful ACTH suppressors (dexamethazone) been used.

If the co-existence of Addison's disease and Graves' disease is more than a coincidence, the mechanism of the interdependence is not clear.

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HYPOPHYSECTOMY IN ACROMEGALY

To the Editor:

Surgical treatment of acromegaly has on the whole been disappointing. In 1909 Cushing (1) described a case of this disease in a young man with severe asthenia and muscular weakness in whom a partial hypophysectomy had been successful. As early as 1908, however, Stumme (2) had reported a similar case in which an operation performed by Hochenegg had been no less successful. Cushing predicted in his paper that pituitary surgery would become accepted in the treatment of acromegaly. This was a reasonable assumption to make, since the condition is a manifestation of abnormal endocrine function induced by a circumscribed benign tumor.

Nevertheless, the surgical treatment of acromegaly has made little progress during the half century that has elapsed since the publication of these papers. Cushing fully realized that normal pituitary tissue had to be preserved, or else hypophysectomy would merely replace acromegaly by a still graver condition—panhypopituitarism. After palliative operations for acromegaly, the disease has often progressed, usually because the tumor could not be completely removed. A general impression of the results may be obtained from Henderson's (3) follow-up of Cushing's patients with acromegaly and also from Bakay's (4) study of Olivecrona's cases. Although partial improvement has occurred, it appears that significant regression of the manifestations of acromegaly has been exceptional.

Conservative treatment—predominantly by x-ray therapy—has generally been recommended for acromegaly. Suprasellar extension of the adenoma has been considered the main indication for neurosurgery. That irradiation therapy often has proved effective and is a valuable therapeutic aid cannot be doubted. It is equally certain, however, that the disease not infrequently resumes its progressive course after an initial remission, and that all signs of activity rarely disappear.

In 1951 Luft and co-workers (5, 6) demonstrated the feasibility of total hypophysectomy and of maintaining the patients with simple substitution therapy. Consequently the question of a more radical removal of the pituitary tumor in acromegaly was revived. We have developed a transantrosphenoidal approach for hypophysectomy (7) which makes total removal of the pituitary gland a reasonably safe procedure. Since the fall of 1955 this method has been adopted in the treatment of 10 cases of acromegaly, the aim being complete removal of the tumor, and thus the termination of the disease. In 4 of these cases, the subsequent development of panhypopituitarism indicated that all normal pituitary tissue must have been removed in addition. All the patients made good recoveries. Meningitis complicated 1 case, but was rapidly controlled by antibiotics.

Whereas acromegaly that had failed to respond satisfactorily to irradiation therapy was the indication for surgery in most of these patients, some of them were operated on without undergoing prior irradiation treatment. With 1 exception, these patients have been followed for periods ranging from three months to two and a half years. All signs of pituitary overactivity have disappeared and the soft-tissue manifestations of the disease have shown significant regression. Indeed, one would never suspect that some of these patients had had acromegaly. Disabling headache is no longer present. One of these patients will now be described more fully. This case is considered particularly interesting because severe diabetes vanished after the operation and did not return.

CASE REPORT

Mr. B.A., a businessman aged 38, was admitted to Sahlgrenska Sjukhuset for treatment of acromegaly in July 1956. His chief complaints were periods of intense headache which impeded him in his work, attacks of profuse sweating, progressive muscular weakness, vertigo and impotence. The tongue had enlarged so that it interfered with speech. The interdental spaces were growing wider in both jaws. About two years previously, diabetes had developed, requiring management with insulin. The patient's appearance, mainly because of swelling of the soft tissues, was typical of acromegaly. Extensive crops of xanthomatous eruptions were present on the legs and buttocks. The blood pressure was normal and no ocular abnormalities were found. With 40 I.U. of regular insulin thrice daily he excreted large amounts of sugar, but there was no ketonuria. Relevant laboratory data are presented in Table 1. X-ray examination of the skull showed that the sella turcica was slightly enlarged, but the encephalographic tracings were normal.

In view of the rapid progression of the acromegalic changes and the severity of the metabolic disturbances, surgical treatment was advocated. Complete hypophysectomy, using our transantrosphenoidal approach, was performed in August 1956. Microscopic

TABLE 1. LABORATORY DATA IN A CASE OF ACROMEGALY, BEFORE AND AFTER HYPOPHYSECTOMY

	Before surgery	Two months after surgery
Blood glucose (mg./100 ml.)	200–400 (120 I.U. insulin)	100–110
Urinary glucose (Gm./24 hrs.)	100–200	0
Glucose tolerance (Exton-Rose); blood glucose (mg./100 ml.)	—	110–180–150
BMR(%)	+40	−15; −20
Thyroidal 24-hr. uptake of I^{131} (% dose)	33	5
Serum protein-bound iodine (μg./100 ml.)	7.5	2.7
Serum cholesterol (mg./100 ml.)	400	200
Urinary 17-ketosteroids (mg./24 hrs.)	12	1

examination of the pituitary revealed an acidophilic adenoma. The patient made a good recovery. Insulin was withdrawn on the first postoperative day and has not been needed since. Glycosuria diminished after a week or two and vanished within a month; thereafter the patient was normoglycemic at every examination. During the first postoperative week, the hands and feet became appreciably smaller and the tongue reverted to normal size and no longer interfered with speech. Headaches vanished and did not return. As soon as the serum cholesterol level had become normal, the diabetic xanthomatosis disappeared. Polyuria, which commenced after the operation, necessitated administration of Pitressin for some months. The laboratory data two months after the operation are shown in Table 1.

The patient has been in good condition. At the time of the latest follow-up examination in December 1958—twenty-eight months after the operation—he felt completely well and was working fulltime. He was receiving 12.5 mg. of cortisone acetate twice daily, 0.1 mg. of sodium thyroxine twice daily, and a long-acting testosterone product by injection twice monthly. Pitressin substitution therapy was no longer required. There were no headaches or attacks of sweating. Potency was practically normal and muscular power was adequate. The patient stated that his teeth had moved closer together, but whether they had done so could not be established. On examination the acromegalic changes were found to have subsided to such an extent that the diagnosis scarcely could be made by a physician without knowledge of the past history. The patient was still normoglycemic.

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DEXAMETHASONE THERAPY IN THE ADRENOGENITAL SYNDROME—A COMPARATIVE STUDY*

TO THE EDITOR:

Since the advent of modern adrenal corticoid therapy in 1949 (1), there has been a continued search for newer, more effective synthetic substitutes for cortisone and hydrocortisone which might also manifest fewer of the undesirable side-effects of the parent compounds. As a result, numerous manipulations in saturation, halogenation, and substitution of organic radicals on the basic cyclopentanophenathrene steroid nucleus have yielded a variety of compounds, most of which differ in clinical effects primarily only in one facet—their equivalent therapeutic dose levels. The outstanding exception to this generalization is 9α-fluorohydrocortisone, which has predominantly mineralocorticoid effects. Other variations of the basic steroid nucleus have produced compounds which are glucocorticoid, anti-inflammatory, and pituitary (ACTH) inhibiting in action. The choice of steroids available for clinical use now includes prednisone, prednisolone, 6-methyl prednisolone, 9α-fluoro-16α-hydroxyprednisolone (triamcinolone), and the most recent one—9α-fluoro-16α-methylprednisolone (dexamethasone).

Previous studies have shown that the therapeutic potency and dose equivalency of corticosteroids can be measured by their effectiveness in suppressing the excessive 17-ketosteroid excretion levels in patients with the adrenogenital syndrome due to adrenocortical hyperplasia (2). Since the excessive urinary 17-ketosteroid levels are a manifestation of unopposed secretion of ACTH from the pituitary (3–7), a decrease in 17-ketosteroids as a result of therapy would reflect the degree to which the administered dosage of a test substance suppressed the excessive ACTH stimulation of the adrenal glands.

Using a group of patients with the adrenogenital syndrome, in whom urinary 17-ketosteroids and/or pregnanetriols were elevated, a comparison has been made between prednisone and dexamethasone regarding the dosages effective for ACTH inhibition. Prednisone was selected as a standard for comparison, since experience with this steroid has shown that 7.5 to 10 mg. a day given in fractional doses every six to eight hours is the aver-

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TABLE 1. COMPARABLE EFFECTIVENESS OF PREDNISONE AND DEXAMETHASONE IN INHIBITION OF ACTH IN PATIENTS WITH THE ADRENOGENITAL SYNDROME, AS MEASURED BY EXCRETION OF 17-KETOSTEROIDS AND PREGNANETRIOL

Patient	Type of adreno-genital syn.*	Age (yrs.)	No steroid therapy		Prednisone therapy			Dexamethasone therapy		
			17-Keto-steroids (mg./24 hrs.)	Preg-nanetriol (mg./24 hrs.)	17-Keto-steroids (mg./24 hrs.)	Preg-nanetriol (mg./24 hrs.)	Daily dose (mg.)	17-Keto-steroids (mg./24 hrs.)	Preg-nanetriol (mg./24 hrs.)	Daily dose (mg.)
1	C	15	29.2	23.4	20.2	—	7.5	8.6	10.4	0.6
					17.7	—	10.0	14.2	9.6	0.75
					18.8	—	10.0	5.3	1.7	0.8
					12.8	—	10.0			
					7.5	—	10.0	2.8	0.3	1.125
					11.4	—	7.5	4.1	0.2	1.2
					8.3	—	12.5			
2	C	28	47.6	57.2	4.9	—	7.5	7.6	2.5	0.75
			20.0	81.3	18.4	—	5.0	9.0	2.1	1.125†
			26.3	41.4	6.1	—	10.0	4.8	1.3	1.125‡
			29.4	55.8	6.6	—	7.5			
3	C	50	15.3	5.8	5.7	0.9	10.0	5.5	—	0.6
			11.9	10.6				4.5	2.1	1.125
			12.8	6.2				4.6	0	1.5
4	P-P	29	20.9	1.8	9.4	0.1	10.0	6.1	1.0	1.2
			29.5	—				3.8	0.1	1.0
			18.6	2.1				7.7	2.5	1.0
5	P-P	22	15.6	1.7	6.3	0.5	7.5	3.3	0.3	1.125
								6.1	0.4	1.125
6	P-P	31	38.4	—	25.5	—	7.5	18.3	2.6	0.8
			33.6	—	14.2	—	10.0	15.8	0.4	1.0
			20.8	0.4	12.5	—	10.0	12.0	1.0	1.0
					11.4	—	10.0	17.2	2.5	1.12
					8.6	—	10.0			
7	C	36	32.9	—	16.6	—	7.5	14.7	0.9	1.125
			42.1	—						
8	C	23	22	—	10.8	—	7.5	10.8	0.2	0.75
			28.3	50.4	8.9	—	7.5	7.0	0.8	1.125
					7.3	—	10.0	15.2	1.0	1.5
9	C	44	12.9	19.1	11.2	—	10.0	11.3	7.9	1.2
			21.6	13.8	9.2	—	10.0	12.3	3.8	1.125
					12.5	—	7.5	14.6	11.3	1.125
					11.3	—	7.5	14.8	5.8	1.125
					8.8	—	7.5	14.4	0.3	1.125
					9.2	—	7.5			
					7.8	—	7.5			
10	C	23	46.2	48.7	15.9	—	10	16.6	50.6	1.0
			98.3	60.8	9.2	0.5	7.5	1.7	0.4	1.0
			61.6	32.0				3.9	0.7	1.25
								4.9	0.3	0.75

* C = Congenital.
P-P = Post-pubertal.
† Results of pregnancy test, positive.
‡ Pregnanediol excretion, 3.6 mg. per 24 hours in second month of gestation.

TABLE 1—(*Continued*)

11	P-P	19	20.9	1.8	4.2	0.1	7.5	5.4	0.9	1.0
12	C	7	11.9	6.9				2.4 4.4 5.8	0.1 0.1 3.0	1.2 1.2 0.6
13	P-P	43	22.5 30.2 19.3	— — 0.9	10.3 6.8	— —	7.5 10.0	7.7 9.4	0.3 0.4	0.8 0.8
14	P-P	32	17.4 16.7	— 1.6				7.7	1.1	1.0
15	P-P	25	17.5	1.7				5.5	0.6	0.75
16	P-P	38	17.0 14.5	— 1.2	7.0	0.8	7.5	7.2 8.9	0.7 0.5	0.75 0.375

age dosage range required for the effective biochemical and therapeutic control of patients with the adrenogenital syndrome. Urinary steroid levels become normal, progressive virilization is halted, and normal feminization is allowed to take place. Table 1 shows the results of prednisone and dexamethasone therapy in varying dosage on urinary steroid patterns in a series of patients with both biochemical and clinical evidence of the adrenogenital syndrome. Eight of the 16 patients, ranging in age from 7 to 44 years, were known to have the classic congenital type of the disorder, and the remaining 8 were apparently normal until puberty or later, when evidences of increased androgenicity developed. The term, "post-pubertal type of adrenogenital syndrome," was applied to those patients with menstrual irregularities and ovulatory defects, with or without hirsutism, in whom urinary 17-ketosteroids were elevated above the normal range in this laboratory (8 to 14 mg. per twenty-four hours). These cases differed from the congenital type of the disorder not only in the time of onset of the disease and in absence of developmental stigmata, but also in the failure to show an increase in pregnanetriol excretion. Thus, although the post-pubertal type may be a biologic variant of the congenital type of the adrenogenital syndrome as originally described, the biochemical and clinical responses are identical. From Table 1 it can be seen that whereas 7.5 to 10 mg. of prednisone daily was the effective therapeutic dosage range for that steroid, only 0.75 to 1.125 mg. of dexamethasone daily was required to achieve a comparable biochemical response. Both preparations were administered three to four times daily in divided dosage.

From these data, it can be inferred that dexamethasone has 7 to 10 times the potency of prednisone with respect to inhibition of ACTH. At the dosage levels used, no undesirable side-effects of steroid therapy were noted. Three patients not included in this series, who were being treated

with dexamethasone for reasons other than therapy of the adrenogenital syndrome, noted insomnia with euphoria which may have been related to the medication.

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AN UNUSUAL CASE OF ADRENAL DYSFUNCTION IN THE MALE

TO THE EDITOR:

Some cases of feminizing tumor of the adrenal have been recently reported (1–2). The present communication concerns a peculiar dysfunction of the male adrenal which apparently is not clearly understood.

CASE REPORT

A 25-year-old male had gynecomastia from the age of 17, a feminine body build, loss of libido and a low sperm count (42,700,000 per ml.; motility 65 per cent).

As seen in Table 1, the urinary excretion of gonadotropin was low; urinary 17-ketosteroids were at the upper limit of normal, with both an absolute and a relative increase in dehydroisoandrosterone; phenolsteroids and pregnanediol were somewhat elevated; and 17-hydroxycorticosteroids and pregnanetriol were normal.

Under ACTH stimulation a further increase of dehydroisoandrosterone took place, and the administration of prednisone (20 mg. per day) caused a rapid and considerable decrease of the urinary 17-ketosteroids, especially dehydroisoandrosterone and the phenolsteroids. After prolonged treatment with prednisone, the gynecomastia was reduced, masculine secondary sexual characteristics became evident, and libido and spermatogenesis were restored (83,300,000 per ml.; motility 85 per cent).

It seems possible that a form of androgenic hyperfunction of the adrenal may appear in the adult male, with characteristics similar to those of congenital adrenal hyperplasia. However, congenital adrenal hyperplasia, in the male, produces precocious isosexual pseudopuberty and is characterized by increased excretion of androsterone. Our patient, on the other hand, exhibited marked feminization and an increase in urinary dehydroisoandrosterone and related steroids.

It is interesting to speculate to what extent the clinical picture is correlated with the hormonal levels.

It has been known for some time that dehydroisoandrosterone has "ambisexual" activity. This steroid is present in both sexes, and has a weak androgenic effect with estrogen-like activity in various animal species. The data on human subjects appear contradictory and inconclusive; it seems, however, that dehydroisoandrosterone has a far weaker androgenic effect than have the other hormonal metabolites such as androsterone, although it has a marked effect on hair growth.

Several instances of adrenal hyperplasia (3) or of adrenal tumors (4–6) have been reported in women with excessive body hair as the only sign of virilism; in these cases there also was a marked increase in urinary dehydroisoandrosterone. We have reported increased dehydroisoandrosterone excretion in female patients who presented, with "idiopathic" hirsutism, no signs of genital dysfunction, no voice changes, and no alterations of body build or mental outlook such as are seen in cases with high levels of androsterone (7).

The effects of excess dehydroisoandrosterone in the male are not well known. Cases have been reported of feminizing tumor with high urinary excretion of androgens in the absence of any signs of masculinization; in these instances the greater part of the urinary 17-ketosteroids was made up by the dehydroisoandrosterone fraction and related steroids (8–10).

TABLE 1

Date	Treatment	24-Hour urinary excretion							Chromatographic fractions of 17-ketosteroids (mg./24 hrs.)			
		17-Ketosteroids (mg.)	Dehydroisoandrosterone (mg.)	17-Hydroxycorticoids (mg.)	Phenolsteroids (μg.)	Pregnanediol (mg.)	Pregnanetriol (mg.)	Gonadotropin (M.U.)	I+II+III	IV	V	VI+VII+VIII
Jan. 30, 1958		15.3	1.68	4.27	36.1	0.96	0.21	5	7.19	2.63	2.34	3.12
Feb. 8, 1958		12.3	1.02									
Feb. 26, 1958		12.4	1.61	2.52	41.2							
Feb. 27, 1958	ACTH	26.1	2.65	9.60	57.0							
Feb. 28, 1958	20 u./day	26.8	3.91	25.20	63.4	1.82	0.14		12.95	4.43	3.58	5.84
Mar. 1, 1958		17.6	2.11	16.40								
Mar. 11, 1958		9.5	0.36	4.27					4.72	1.01	2.16	1.61
Mar. 24, 1958		8.2	0.54	1.61	43.2							
Apr. 30, 1958		9.9	1.12	2.48	38.2			5				
May 1, 1958	Prednisone											
May 7, 1958		0.9	0									
May 14, 1958	20 mg./day	2.8	0									
May 29, 1958		2.8	0		31.6	0.81	0		0.79	1.08	0.64	0.29
June 11, 1958		1.6	0		26.5			20				
July 4, 1958	10 mg./day	3.4	0		13.2				1.02	1.36	0.72	0.30
Sept. 15, 1958		4.4	0		23.4			20				

Methods used	Normal range (in our laboratory) for adult men
17-Ketosteroids: Callow *et al.* (12)	8–13 mg./24 hrs.
Dehydroisoandrosterone: Allen *et al.* (13)	0–0.30 mg./24 hrs.
17-Hydroxycorticoids: Reddy *et al.* (14)	3–6 mg./24 hrs.
Phenolsteroids: Bates & Cohen (15)	10–20 μg./24 hrs.
Pregnanediol: Bongiovanni & Clayton (16)	0–0.5 mg./24 hrs.
Pregnanetriol: Bongiovanni & Clayton (16)	0–0.5 mg./24 hrs.
Gonadotropin: Gorbman (17)	20–40 M.U./24 hrs.
Chromatographic separation of 17-KS: Johnsen (18)	fractions I+II+III, 23–46%
	IV, 34–43%
	V, 21–36%
	VI+VII+VIII, 0–8%

These observations support the view that excess excretion of dehydroisoandrosterone may be associated with evident feminization in the male.

In our case also there was concomitant hyperproduction of estrogens by the adrenal and diminished testicular function; dehydroisoandrosterone appears to inhibit pituitary gonadotropins.

Perloff and Hadd (11) recently reported a case which is similar to ours in various aspects, namely, impotence and altered spermatogenesis due to a fall in gonadotropins; the urinary 17-ketosteroids were comprised mainly of androsterone. However, the authors achieved their chromatographic

results after enzymatic hydrolyis of the steroids with β-glucuronidase. Since dehydroisoandrosterone is excreted mainly as sulphate, the greater part of it would not have been detected with this method.

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February 18, 1959

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URINARY 17-KETOSTEROID EXCRETION IN CASTRATED AND INTACT MEN

To the Editor:

The recent publication by Hamilton, Bunch and Mestler (1) in this Journal dealing with urinary 17-ketosteroid (17-KS) and 17-hydroxycorticosteroid (17-OHCS) excretion in castrated and intact men is of interest to us because of studies (2) reported approximately one year previously from this laboratory on urinary 17-KS excretion in castrated and intact male subjects who were inmates of the same institution[1] from which the subjects for the study by Hamilton *et al.* were selected. The groups studied by Hamilton and by us contained many of the same individuals.

We reported urinary 17-KS excretion in these institutionalized feebleminded subjects as part of a study of the effects of castration; the data also included determinations of serum lipids, lipoproteins, glycoproteins and urinary gonadotropin. The methods employed by Hamilton *et al.* and by us for determination of 17-KS utilized the Zimmermann reaction for color development. We were assisted in various aspects of these studies by L. D. Bunch and R. Imagawa, both of whom had been associated with Hamilton in one or more of the studies which the latter had undertaken at the Kansas institution. Acknowledgment was made by us of the assistance provided by Bunch, and Imagawa was a co-author of our publication (2).

It is surprising, then, to note that Hamilton, Bunch and Mestler failed to acknowledge the previously published observations from this laboratory on the urinary 17-KS excretion of these eunuchs and their intact fellow inmates. Priority is also claimed by them for observations previously reported by us. We invite attention to the following points:

[1] Winfield State Training School, Winfield, Kansas.

1) Hamilton states in his introduction: "Previous investigations of the influence of castration upon titers of urinary 17-KS have been limited to small groups of subjects (3, 4), mostly old or ill with cancer, and the excretion of 17-OHCS after orchiectomy has apparently not been studied."

Comment: Our report included mean values for urinary 17-KS excretion in 24 castrated and 19 intact male feeble-minded subjects ranging in age from 23 to 79 years, who were otherwise in apparent good health. The study by Hamilton *et al.* involved 52 castrated and 31 intact male feeble-minded subjects ranging in age from 24 to 81 years. The subjects were chosen from the same institution in both studies.

2) Regarding 17-KS excretion, Hamilton states: "Mean and median values tended to be lower at all ages in the castrated men than in the intact controls, but the difference was of questionable significance ($P = .05$) in the series as a whole." It is also stated "Because of the elevated titers of 17-KS in some eunuchs and low titers in the control series of feeble-minded males, the difference in steroid excretion between these castrated and intact males is less than in previous reports."

Comment: In the publication from this laboratory it is stated: "The 17-ketosteroid excretion per 24 hours did not differ significantly between the 2 groups for any age category." The percentage difference in steroid excretion cited by Hamilton is similar to the percentage difference which may be determined from the 17-KS values given in Table 3 of our publication.

3) Hamilton states further: "With advancing age, the titers of 17-KS underwent a reduction that approached significance in both castrate ($P = .03$) and intact ($P = .04$) control groups. . . . The new finding, that a reduction in 17-KS with aging occurs in eunuchs, is proof that extratesticular tissues are involved in this phenomenon."

Comment: In the publication from this laboratory it is stated: "Aging appeared to be associated with diminishing urinary 17-KS, a phenomenon previously described by Venning and Kazmin" (5). The mean 17-KS excretion in castrated subjects between the ages of 31 and 50 years was reported as 9.7 mg. per twenty-four hours, with a standard error of 0.7. The excretion in castrates 51 years of age or more was 5.3 mg., with a standard error of 1.0

4) In the paper by Hamilton it is stated: "Titers of 17-KS and of 17-OHCS, either in absolute values or relative to body weight, were not related significantly to the subject's age at the time of castration or to the duration of the castrate state."

Comment: In the publication from this laboratory the following observation is made: "No correlation was noted between urinary 17-KS ex-

cretion and the subject's age at castration, the concentration of high or low density lipoproteins or their ratio."

We believe that these previously published observations from this laboratory should be brought to the attention of the reader.

ROBERT H. FURMAN, M.D.
R. PALMER HOWARD, M.D.

The Cardiovascular Section of the
Oklahoma Medical Research Foundation,
and the Department of Medicine,
University of Oklahoma School of Medicine,
Oklahoma City, Oklahoma,
May 26, 1959

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The Endocrine Society

THE 1960 ANNUAL MEETING

The Forty-Second Annual Meeting of The Endocrine Society will be held in the Eden Roc Hotel, Miami Beach, Florida, Thursday, Friday and Saturday, *June 9, 10 and 11, 1960.*

The Chairman of Local Arrangements is Dr. Kenneth Savard.

Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily and in addition there will be simultaneous afternoon sessions. The annual dinner is scheduled for Friday, June 10, at 7:30 P.M. preceded by cocktails at 6:30 P.M.

All members are urged to make their hotel reservations immediately. The Eden Roc will hold 250 bedrooms and the adjoining hotels, the Montmartre Hotel and Lucerne Hotel, will hold 75 rooms each for members until May 1, 1960, after which time the hotel will not guarantee further reservations. Therefore it is imperative that you make your reservations early, directly with the hotel, advising them of time and date of arrival and departure. If you plan on remaining through June 12 for the American Medical Association and other meetings, it is imperative that your reservation be made from June 12 on, directly through the A.M.A. Housing Bureau, c/o Miami Beach Convention Bureau, 1700 Washington Avenue, Miami Beach, Florida, since no reservation beyond June 12 can be accepted directly by the hotels. Make your reservations early and avoid disappointment. Hotel reservation card will be mailed to members shortly after January 1, 1960.

Final program, membership card and advance registration forms will be sent on May 1, 1960 to members whose current dues have been paid.

Those wishing to present papers, which will be strictly limited to ten minutes, should send an original and 4 copies of the title and abstract to the Vice-President, Dr. Robert H. Williams, University of Washington School of Medicine, Seattle 5, Washington, *not later than February 15, 1960.* It is imperative that the abstracts be informative and complete with results and conclusions—not a statement that these will be presented at the meeting—in order that they may be of reference value and suitable for printing in the program. The reading and processing of approximately 200 abstracts submitted each year and compiling of the program from these involves a tremendous amount of time and effort on the part of the

Program Committee. THE COUNCIL REQUESTS THAT AUTHORS ADHERE STRICTLY TO THE FOLLOWING RULES WHEN SUBMITTING ABSTRACTS; OTHERWISE THEY CANNOT BE CONSIDERED:

1. IT IS ASSUMED THAT ABSTRACTS SUBMITTED FOR THIS PROGRAM HAVE NOT AND WILL NOT BE SUBMITTED ELSEWHERE.
2. Abstracts may not exceed 200 words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formula cannot be used.
3. The title heading must be arranged as follows:
   Line 1. Title, not to exceed 15 words;
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be written after his name.
   Line 3. Institution of origin and city in which institution is located.
4. Authors who wish receipt of their abstracts acknowledged should enclose self-addressed post card with the title of the abstract noted thereon.
5. Authors are also to enclose 3×5 inch filing cards, one for each author, giving his name and initials in the upper right-hand corner, and the complete title of the abstract with all the authors listed in the center of the card. There should be a second card enclosed listing the title, all authors, and the mailing address of the senior author. This is necessary for indexing. Senior authors will be notified of the acceptance of their papers for the final program.
6. The body of the abstract, typed double space, should follow the heading. The original copy should be on bond paper. The original and 4 copies should be forwarded to Dr. Williams.
7. Abstracts should be letter perfect, since there will be no opportunity for proof reading by the authors.

THE 1960 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

FRED CONRAD KOCH AWARD

In 1957 a substantial legacy was bequeathed to the Society by the late Elizabeth Koch for the purpose of establishing the Fred Conrad Koch Memorial Fund in memory of her late husband, Distinguished Service Professor of Physiological Chemistry at the University of Chicago and pioneer in the isolation of the androgens. This is the highest honor of The Endocrine Society and is represented by a medal known as the Koch Medal of The Endocrine Society. The medal, as well as an honorarium of $3,500, is to be given annually to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

This Award was first presented in 1959, to Dr. W. W. Swingle. The Koch Medal has replaced the Medal of The Endocrine Society which was established in 1954 and presented to Dr. Carl R. Moore in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957 and Dr. I. L. Chaikoff in 1958. The Medal of the Endocrine Society replaced the E. R. Squibb Award which was formerly the highest honor bestowed by the Society. Past recipients of the Squibb Award were Dr. George W. Corner in 1940, Dr. Philip E. Smith in 1941, Dr. Fred C. Koch in 1942, (no award in 1943), Dr. E. A. Doisy in 1944, Dr. E. C. Kendall in 1945, Dr. Carl G. Hartman in 1946, Drs. Carl F. and Gerty T. Cori in 1947, Dr. Fuller Albright in 1948, Dr. Herbert M. Evans in 1949, Dr. C. N. H. Long in 1950, Dr. J B. Collip in 1951, Dr. James H. Means in 1952 and Dr. David Marine in 1953.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—

Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer; 1959—Dr. Gordon L. Farrell. Prior to 1952 the Award was $1,200. It has now been increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE UPJOHN SCHOLAR OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. A grant has been made available through the generosity of the Upjohn Company, and will be awarded to an established investigator or teacher in the field of endocrinology who wishes to extend the opportunities for work either in this country or abroad.

The award will not exceed $2,500 annually and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Fred Conrad Koch Award, the Ciba Award, and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be

obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *October 15* each year.

Proposals for appointments as a Scholar of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *October 15* each year.

ELEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE UNIVERSITY OF CALIFORNIA SCHOOL OF MEDICINE AND DEPARTMENT OF CONTINUING MEDICAL EDUCATION

SAN FRANCISCO, CALIFORNIA

November 9 through 13, 1959

For information concerning the program and registration, write to *Seymour M. Farber, M.D.*, Assistant Dean in charge of Continuing Medical Education, University of California Medical Center, San Francisco 22, California.

The registration fee is $100 payable to the Regents of the University of California. Residents and Fellows will be admitted for a reduced fee of $30. Those wishing to reserve rooms should communicate directly with the Whitcomb Motor Hotel, 8th and Market St., San Francisco.

(For program, see the June issue of the Journal)

FIRST INTERNATIONAL CONGRESS OF ENDOCRINOLOGY

The First International Congress of Endocrinology will be held in *Copenhagen, Denmark, July 18–23, 1960.*

United States members of the International Executive Committee are Dr. Warren O. Nelson and Dr. Leo T. Samuels, Dr. Gregory Pincus is

Chairman of the Program Committee. The tentative program consists of ten symposia covering the general field of endocrinology plus round-table discussions and a number of submitted papers. Those desiring to submit papers should immediately contact Dr. Gregory Pincus, Worcester Foundation, Shrewsbury, Massachusetts. There will be a number of interesting scientific and technical exhibits.

In order to facilitate travel arrangements to the Copenhagen Convention, the Council of The Endocrine Society at its San Francisco meeting designated THOMAS COOK & SON as our authorized travel agents. They will in turn offer especially designed All Expense Paid Tours to Europe, including hotel reservations for the convention, as well as offering independent itineraries for any of our members who desire to travel separately by air or by steamship.

Registration is open to anyone interested in endocrinology at an approximate fee of $28 U.S. Members of the families of those attending may register as associates at an approximate fee of $14 U.S., fee to be paid at the time of registration or in advance to the American Express Company, which has been designated by the Executive Committee of the International Congress as official financial and travel agent. There will be a number of social functions and other entertainment provided for all registrants.

As you all know, European travel is unusually heavy during July and it is suggested that those contemplating attending the Congress should start making tentative reservations at the earliest possible moment by filling in and returning the card recently mailed to members, to THOMAS COOK & SON's special representative—Harry E. Kornbaum, Transportation Chairman, Rainbow Travel Service, 2817 Classen Boulevard, Oklahoma City 6, Oklahoma, who will correspond directly with each member about his individual travel arrangements to and from the Convention, at the same time keeping our National Officers fully informed of all travel arrangements of its members. Completion of the card CARRIES NO OBLIGATION OR COMMITMENT ON YOUR PART. Send it in today for the complete travel arrangements for our Society.

The general expenses of a meeting of this magnitude are quite large and The Endocrine Society has made a substantial contribution from its treasury for organizational purposes. U.S. pharmaceutical firms have also contributed very generously. However, sufficient funds are not as yet available to assure the success of this meeting; therefore, *the committee suggests that each of our members make voluntary contributions to this fund.* Please make your checks payable to: The Endocrine Society—First International Congress of Endocrinology. Mark it *Contribution* and mail it to the office of the Secretary-Treasurer, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

PROGRAM

The program will consist of ten Symposia and a Round Table Discussion, and of groups of shorter (ten-minute) communicated papers. Speakers at the Symposia and the Round Table Discussion are invited by the Congress. Communicated papers may be submitted by investigators in the field of endocrinology, and titles and abstracts are invited. Forms for registration in the Congress, along with forms for the submission of abstracts of short communications, may be obtained by writing to the Congress Secretariat: Dr. Svend G. Johnsen, Hormone Department, Statens Seruminstitut, Copenhagen S, Denmark. The final date for the submission of such abstracts is *December 31, 1959*. The official languages of the Congress are English, French, German and Spanish. A volume containing advance abstracts of symposium contributions and the short communications (in one of the official languages and translated into Interlingua) will be distributed to members on registration at the Technical University of Denmark in Copenhagen. Titles and abstracts of communicated papers should not be sent to the Program Committee, but the Program Committee will review and arrange for the presentation of such papers in appropriate Congress sessions.

GREGORY PINCUS, *Chairman*,
Subcommittee on Program

APPLICATIONS FOR TRAVEL ALLOTMENTS

The Endocrine Society has obtained funds to provide a limited number of allotments in support of travel to the Congress for scientists residing in the United States and Canada who may require such assistance. Individual allotments will not exceed $750.00 each.

Application for travel allotment should be submitted in duplicate in the form of a letter giving information on age, training, publications, academic or professional title and society affiliation. Applicants who plan to submit papers for presentation at the Congress should include the proposed title of each paper. Such letters should be submitted *before November 15, 1959* and should be addressed to Dr. H. H. Turner, Secretary, The Endocrine Society, 1200 North Walker, Oklahoma City 3, Oklahoma. Applicants will be responsible for their own passports, registration, travel arrangements and hotel reservations.

Application for assistance towards travel expenses are entirely separate from applications for registration for the Congress and for submission of papers, information on which may be obtained by writing to The Secretariat, Hormonafdelingen, Statens Seruminstitut, Copenhagen.

The American Goiter Association

THE 1960 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in *London, England, July 5–9, 1960,* in the Great Hall of the Royal College of Surgeons. This meeting, co-sponsored with the London Thyroid Club, will be the Fourth International Goiter Conference. For further information write Dr. Selwyn Taylor, 3 Roedean Crescent, Roehampton, London, S.W.15, England.

JOHN C. MCCLINTOCK, *Secretary,*
149½ Washington Avenue,
Albany 10, N.Y.

SUBMISSION OF ABSTRACTS FOR THE PROGRAM OF THE FOURTH INTERNATIONAL GOITER CONFERENCE

Abstracts of American papers to be considered for presentation at the Fourth International Goiter Conference, London, England, July 5–9, 1960, should be sent to Dr. J. E. Rall, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda 14, Maryland. Abstracts from all other countries are to be sent to Dr. Selwyn Taylor, 3 Roedean Crescent, Roehampton, London, S W.15, England.

All American abstracts must be received by Dr. Rall *by December 1, 1959.* They should not exceed 400 words and should be submitted in quintuplicate. Presentation of papers will be limited to fifteen minutes and a copy of the final paper must be sent to the London Secretary two weeks prior to the meeting.

THE VAN METER PRIZE AWARD FOR 1960

The American Goiter Association again offers the Van Meter Prize Award of $300.00 to the essayist submitting the best manuscript of original and unpublished work concerning "Goiter—especially its basic cause." The studies so submitted may relate to any aspect of the thyroid gland in all of its functions in health and disease. The Award will be made at the Fourth International Goiter Conference in London, England, July 5–9, 1960, where a place on the program will be reserved for the winning essayist if he can attend the meeting. For 1960, the recipient of the Award will receive consideration for an award of a travel honorarium.

The competing essays may cover either clinical or research investigations, should not exceed 3,000 words in length and must be presented in English. Duplicate typewritten copies, double spaced, should be sent to

the Secretary, Dr. John C. McClintock, 149½ Washington Avenue, Albany 10, New York, *not later than January 1, 1960.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

CONGRESS OF THE PAN AMERICAN MEDICAL ASSOCIATION

The Congress of the Pan American Medical Association will meet in Mexico City, *May, 2–11, 1960.* There will be a Section on Endocrinology. Authors wishing to present papers are invited to submit titles and an abstract in triplicate, of not more than 250 words. Abstracts must be informative and must include results and conclusions. Please indicate, on one copy of the abstracts, whether you will need a slide projector, and what size ($3\frac{1}{4}\times4$ or 2×2). Papers will be limited to 15 minutes. Technical information regarding hotel reservations, transportation, etc. will be announced by the Pan American Medical Association shortly. Please send abstracts to the program chairman Dr. Karl E. Paschkis, Jefferson Medical College, Philadelphia 7, *not later than December 31, 1959.*

INTERNATIONAL ACADEMY OF PATHOLOGY

An overseas meeting of this association will be held at the Royal College of Surgeons of England, Lincoln's Inn Fields, London W. C. 2, England, from *June 20 to June 24, 1960.* The presentations will consist of proffered papers on June 20; on June 21 a slide seminar on Proliferative Lesions of the Breast with Professors Ch. Oberling of France, R. W. Scarff of Great Britain and Lauren Ackerman of the United States as Moderators; on June 22 an all-day Symposium on the Patho-physiology and Pathology of the Kidney by invited speakers; and on June 23 and 24 a Joint Meeting of the American and British Pathologists with a Symposium on Thyroiditis and Cancer of the Thyroid. Scientific exhibits will be accepted and visits to Research Centres will be arranged. In addition, there will be a social program for pathologists and their wives. For detailed information, please write to either:

Dr. F. K. Mostofi,
Secretary,
International Academy of Pathology,
Armed Forces Institute of Pathology,
Washington 25, D. C., U. S. A.

or

Professor George J. Cunningham,
Secretary,
London Meeting of the International Academy of Pathology,
Royal College of Surgeons of England,
London W. C. 2,
England.

SYMPOSIUM ON FUNDAMENTAL CANCER RESEARCH

The University of Texas M. D. Anderson Hospital and Tumor Institute. Houston, Texas, announces the Fourteenth Annual Symposium on Fundamental Cancer Research—"Cell Physiology of Neoplasia"—to be held *February 25–27, 1960*. The general scope of the program includes morphology of cells, behavior of organelles, submicroscopic structures, cytochemistry, growth phenomena and biochemical properties of cells. Assisting The University of Texas M. D. Anderson Hospital is the University of Texas Postgraduate School of Medicine, and contributing to the support of the meeting are The American Cancer Society, the Texas State Department of Public Health, and the National Cancer Institute, United States Public Health Service. Further information may be obtained by writing: Editorial Office, The University of Texas M. D. Anderson Hospital, Texas Medical Center, Houston 25, Texas.

NEW CLINICAL CENTER STUDY ON THE STEIN-LEVENTHAL SYNDROME

An investigation of the role of the adrenal gland in the Stein-Leventhal syndrome has been initiated at the Clinical Center, National Institutes of Health. Salient features in this syndrome include oligomenorrhea, hirsutism and polycystic ovaries.

The cooperation of interested physicians in referring laparotomy-proven cases is invited. Referral letters or phone calls will receive prompt attention and should include detailed medical information about the patient.

Accepted patients will be studied for varying periods up to several weeks. Upon completion of their study, patients will be returned to the care of their referring physician, who will also receive a detailed narrative summary. In some instances it may be desirable to arrange for occasional followup visits to the Clinical Center. These would supplement rather than substitute for visits to the patient's own physician.

Physicians interested in the possibility of referring patients should write or telephone:

J. E. Rall, M.D.,
Chief, Clinical Endocrinology Branch,
National Institute of Arthritis and Metabolic Diseases,
Bethesda 14, Maryland
(OLiver 6-4000, Ext. 4181)

or

Saul W. Rosen, M.D.,
Clinical Associate,
National Institute of Arthritis and Metabolic Diseases,
Bethesda 14, Maryland
(OLiver 6-4000, Ext. 2936)